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miR-141-3p Suppresses Expression of Androgen Receptors and Functions as a Tumor Suppressor Gene in Prostate Carcinogenesis

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Abstract

Background: Prostate cancer (PCa) is a leading cause of tumor mortality in Western societies. In China, the PCa mortality rate is increasing yearly. Androgen receptors (ARs) and microRNAs (miRNAs) play central roles in prostate carcinogenesis and progression. **Methods:** To characterize the underlying molecular mechanisms, we compared the miRNA profiles of early PCa (G ≤ 7), advanced PCa (G > 7) and non-tumor prostate tissues using deep-sequencing. The target genes of differentially expressed miRNAs were predicted by bioinformatics analysis and confirmed by luciferase reporter assays and Western blot (WB) and quantitative reverse transcription-PCR (qRT-PCR) analyses. Finally, we performed *in vitro* functional studies by inducing or inhibiting miR-141-3p expression using an artificial mimic or inhibitor. **Results:** A computational search implicated the open reading frame (ORF) of AR mRNA as a potential miR-141-3p target site. The qRT-PCR, WB and luciferase reporter assays revealed a reverse regulatory effect of miR-141-3p on AR. Mutation of the potential miR-141-3p binding site in the AR ORF resulted in a loss of responsiveness to the corresponding miRNA. Moreover, miR-141-3p expression levels were unchanged in early PCas, but were obviously increased in advanced PCas. MiR-141-3p overexpression inhibited RWPE-1 cell proliferation, mobility, and prohibited the entry of cells into the G2-S-M phase; miR-141-3p inhibition had the inverse effects. At the same time, we tested miR-141-3p's functions in PC-3 and VCaP prostate cancer cell lines. **Conclusions:** Taken together, our results indicate that miR-141-3p targets AR and its downstream signaling pathways, and functions as a tumor suppressor miR in PCa carcinogenesis by suppressing cell growth and mobility, but the effect is not significant in maglinant PCas. MiR-141-3p is implicated as a novel therapeutic target for early PCa.

Keywords

Prostate Cancer, miR-141-3p, Androgen Receptor, Carcinogenesis

1. Introduction

Prostate cancer (PCa) is the most common malignancy of the male genitourinary tract, and the second leading cause of cancer deaths among males in Western societies [1]. In the United States in 2013, it was estimated that 238,590 new PCa cases were diagnosed with 29,720 attributable deaths [2]. Androgen receptors (ARs) are one of the most important nuclear transcription factors among the steroid hormone receptor superfamily of genes. Normal prostate growth and development, prostate carcinogenesis, and castration-resistant progression of PCa are dependent on AR expression and function. Alterations in AR structure, expression and signaling could have a defining role in PCa progression. AR is translocated to the nucleus in a dimerized form and regulates gene expression by binding to specific hormone response elements [3]. In the early stages, PCa depends on androgens for growth; therefore, the most effective systemic treatment for this hormone sensitive cancer is androgen deprivation therapy. However, the greatest problem associated with this approach is that, after hormone treatment, the tumor inevitably progresses from an androgen-dependent (AD) form to an incurable castration-resistant (CR) form. Many of these AR-regulated genes are key regulators of prostate development and maintenance. AR signaling is also critical to the initiation and progression of PCa.

MicroRNAs (miRNAs) are small regulatory RNAs that target mRNAs and cause mRNA cleavage and/or translational suppression. They may function as oncogenes or tumor suppressors. Aberrant miRNA expression is closely associated with growth, development, invasion, metastasis, and prognosis of various cancers, including PCa. A number of miRNAs have been reported to be upregulated or downregulated in human PCa and are used as biomarkers for diagnosis, prognosis, and classification. MiR-141-3p is one of these upregulated miRNAs in PCa cells and upregulation of miR-141-3p has been demonstrated to be a biomarker for the disease [4] [5] [6] [7] [8]. Nevertheless, the mechanism by which aberrant miR-141-3p expression contributes to prostate tumorigenesis is still not fully understood.

Several miRNA profiling studies in PCa have been reported, but the results regarding the deregulation of miRNAs are highly inconsistent. We have previously reported deregulation of miRNAs in PCa revealed by deep-sequencing in a study designed to investigate the expression levels and roles of miR-141-3p in PCa carcinogenesis [9]. Expression levels of objective genes were measured by deep-sequencing and quantitative polymerase chain reaction (qPCR) in a small cohort of patients with localized early PCa (Gleason ≤ 7) and non-tumor control tissues [9]. In the present study, the expression levels of miR-141-3p and the ef-

fects on AR transcriptional activity were estimated by upregulating or downregulating miR-141-3p using its mimic or inhibitor in the human non-malignant prostate epithelial cell line RWPE-1and human malignant prostate cancer cell lines PC-3 and VCaP. There were no differences in miR-141-3p expression in early PCa tissues compared to control benign prostatic hyperplasia (BPH) tissues. MiR-141-3p targeted the open reading frame (ORF) region of AR mRNA resulting in AR translational suppression and mRNA degradation and miR-141-3p expression correlated inversely with AR expression. Moreover, *in vitro* functional studies performed by upregulating or downregulating miR-141-3p expression using an artificial mimic or inhibitor showed that miR-141-3p overexpression suppressed cell proliferation and attenuated mobility in RWPE-1 cells. Our findings not only provide new insights into the mechanisms of prostate tumorigenesis, but also reveal a novel strategy for early PCa therapy.

2. Materials and Methods

2.1. Cell Culture

The immortalized benign prostatic epithelial cell line RWPE-1 and human malignant prostate cancer cell lines PC-3 and VCaP, were obtained from the American Type Culture Collection (ATCC), and maintained in regular medium supplemented with 10% fetal bovine serum (FBS) at 37˚C and under 5% CO_2 in a humidified incubator until 80% - 90% confluence.

2.2. Oligonucleotides and Cell Transfection

MiR-141-3p mimic and inhibitor oligonucleotides (Biomics, Jiangsu China) were used at a 100 nM final concentration in the experiments. RWPE-1 cells were plated in 12-well plates at 1.5 - 2.5 × 10^5 cells per well, grown for 24 h at 70% confluence, and then switched to antibiotic free media prior to transfection. Next, 5 μL oligos and 4.8 μL X-tremeGENE 9 DNA Transfection Reagent (Roche, Basel Switzerland) were added individually to 100 μL Opti-MEM (Invitrogen, Carlsbad USA), and then incubated for at least 20 min before being added to the cultured cells. The overexpression and inhibition of miR-141-3p were achieved by transfection of commercial mature miRNA and antisense miRNA with an appropriate miRNA control. The cells were incubated with the aforementioned oligonucleotides after 48 - 72 h. After transient transfection, total RNA extraction, genetic and functional characteristics were analyzed by qRT-PCR and WB.

2.3. RNA Isolation and qRT-PCR Analysis

For quantitative expression analyses of mRNA and miRNA, total RNAs were isolated from Formalin-Fixed and Parrffin-Embedded (FFPE) prostatic tissues (Table S1 and Table S2) or cultured cells using Trizol reagent (Life Technologies, Carlsbad USA) according to the manufacturer's instructions. The RNA concentration and purity were measured with a Nanodrop 2000 (Thermo Scien-

tific, Waltham USA). Total RNAs were reverse-transcribed by the M-MLV reverse transcriptase kit (Invitrogen), and residual DNA was removed by treatment with the DNA-free™ Kit (Ambion, Carlsbad USA). qRT-PCR was performed to detect AR mRNA and miR-141-3p levels using SYBR Green Premix DimerEraser (Takara, Japan) on a Roche 480 system. The primers used are listed in **Table S3**. Melting curves were determined following reactions using the following program: 30 s at 95˚C, followed by 40 cycles of 5 s at 95˚C, 30 s at 56˚C, and 30 s at 72˚C, and the melting curve was determined. GAPDH (for mRNA) and U6 (for microRNAs) levels were used as internal controls, and fold changes were calculated by relative quantification ($2^{-\Delta\Delta Ct}$). To minimize experimental variation, tumor and control samples were analyzed on the same reaction plate, and all reactions were measured in triplicate.

2.4. Western Blotting

Transfected cells were lysed in radio-immunoprecipitation assay (RIPA) buffer (Thermo Scientific) supplemented with protease inhibitors (100 mM Tris-HCl at pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1% deoxycholate acid, 0.1% SDS, 2 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 2 mM DTT, 2 mM leupeptin, and 2 mM pepstatin). Lysates were centrifuged at 12,000 rpm for 10 min under 4˚C, and supernatants containing total proteins were collected. Protein concentrations were determined by the BCA method (Beyotime, Jiangsu China), and aliquots of 20 μg protein lysates were separated by SDS-polyacrylamide gel electrophoresis and then transferred to PVDF membranes (GE Healthcare Life Sciences, USA). Membranes were blocked with 5% non-fat milk solution for 2 h. Immunoblotting was performed using an anti-rabbit AR monoclonal antibody (diluted 1:1,000; Abcam, ER179(2)). GAPDH immunoblotting was performed using an anti-rabbit GAPDH antibody (Abcam, EPR6256). The secondary antibody was goat anti-rabbit HRP-linked (Abcam, ab6721), and blots were developed using enhanced chemiluminescence Detection System (Thermo Scientific). Protein levels were quantified using ImageJ2× software and normalized to GAPDH levels.

2.5. Luciferase Reporter Assays

To construct reporter plasmids, a DNA fragment of the AR ORF containing the putative miR-141-3p binding site (5'-GCCATTGAGCCAGGTGTAGTGTG-3') was amplified by PCR from human cDNA. The AR ORF fragment lacking the miR-141-3p binding site (5'-GCCA-------AG-3') was used as negative control. DNA fragments were inserted downstream of the reporter gene of pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega) after *Sal*I and *Xba*I digestion. The sequences and cloning direction of these plasmids were validated by DNA sequencing. For luciferase reporter assay, 293T cells (4×10^4 per well) were seeded into 24-well plates and cultured for 24 h. The cells were then co-transfected with reporter plasmids and 100 nM chemically synthesized miR-141-3p mimic or miRNA negative control (miR-NC). After 48 h, cells were har-

vested and lysed with passive lysis buffer (Promega, Madison USA). Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) on a Fluroskan Ascent FL Microplate Fluorometer (Thermo Electron) following the manufacturers' instructions. Luciferase activities were expressed as the ratio of firefly to Renilla luciferase activity and normalized to the levels detected in control transfections.

2.6. Cell Proliferation Assay

To assess the contribution of miR-141-3p to PCa cell proliferation, transiently transfected RWPE-1 cells were seeded into 96-well plates (3000 cells/well). Cells were incubated for 12 h to allow them to attach to the bottom of the well before the addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis USA) to measure cell growth at different time points (1 d, 2 d, 3 d, 4 d, 5 d and 6 d). According to the manufacturer's instructions, 10 μL of MTT solution was added to the cultured cells, and incubated for 4 h at 37˚C. The supernatant was removed, and 200 μL of DMSO was added to each well to solubilize the water-insoluble purple formazan crystals before the absorbance at 490 nm was measured. Experiments were performed in triplicate.

2.7. Cell Cycle Assay

Cells were plated in 12-well plates (1.5 - 2.5 × 10^5 cells/well) and transfected with the miR-141-3p mimic or inhibitor. After 48 - 72 h, cells were harvested by trypsinization, and 1 × 10^6 cells were used for cell cycle analysis. The cells were washed with PBS, fixed in 70% ice-cold ethanol overnight at 4˚C, then washed with PBS again and incubated with 1 mL staining solution (20 μg/mL propidium iodide; 10 U/mL RNaseA) for 30 min at room temperature. The DNA content was measured by flow cytometry on a FACS Calibur system (Becton Dickinson, New Jersey USA), and cell cycle distributions of the different populations were determined using FlowJo software (Verity Software House).

2.8. Wound-Healing Assay

Transfected cells were cultured to 80% - 90% confluence in 6-well plates. Cell layers were scratched using a 10 μL pipette tip to form wound gaps and then washed twice with PBS. The wound-healing was photographed at different time points (1 d, 2 d and 3 d). Each wound was analyzed by measuring the distance migrated by cells in three different areas. Data are presented as the mean ± standard deviation (SD) for experiments and compared to control miRNA transfected cells.

2.9. Bioinformatics

Potential miR-141-3p targets were predicted and analyzed using the following four publicly available algorithms: RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid), PicTar (http://pictar.mdc-berlin.de/), TargetScan (http://www.Targetscan.org), and

miRanda (http://www.microrna.org/). Conservative analysis was performed by NCBI-blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.10. Statistical Analysis

Statistical analyses of data were performed with SPSS 20.0. All data are expressed as the mean ± SD of at least three separate experiments. All statistical tests were two-sided, and *P*-values < 0.05 were considered to indicate statistical significance. Unless otherwise noted, the differences between groups were analyzed using either the unpaired Student's *t*-test when only two groups were compared or a one-way analysis of variance (ANOVA) when more than two groups were compared.

3. Results

3.1. Aberrant Expression of miR-141-3p in Human Early PCa

We previously investigated small RNA transcriptomes by deep-sequencing technology to evaluate three pooled libraries [9]. A total of 13,896,705, 16,768,094 and 17,184,337 raw sequence reads were produced for the malignant PCa (G > 7), early PCa (G ≤ 7), and BPH groups, respectively. After filtering out low quality reads and trimming off adaptors, 10,494,173, 14,577,221 and 14,614,835 sequence reads were obtained [9]. The normalized counts of sequencing reads (specific miRNA/total sequencing tags in the library) were used to quantify miRNA expression levels among the PCa (G > 7), PCa (G ≤ 7) and BPH groups. The statistical significance (*P*-value) was inferred based on the Bayesian method, which was developed for analysis of digital gene expression profiles. We found a 1.26-fold reduction in miR-141-3p expression in early PCa compared to BPH ($P < 0.05$), while compared to the early PCa samples, the average miR-141-3p level increased by 3.90-fold in advanced tumors ($P < 0.05$) (**Figure 1(a)**). A number of previous reports describe the effects of miR-141-3p overexpression in human PCa [4] [5] [6] [10], although reports of the effects of inhibited miR-141-3p expression in early PCa are rare.

To further validate the sequencing data, we independently chose 32 early PCa, 14 malignant PCa and 16 BPH tissues. Total RNAs were prepared and analyzed by qRT-PCR. We determined whether miR-141-3p downregulation was common in clinical early PCa tissues. The boxplot of qRT-PCR analyses is shown in **Figure 1(b)**. The average miR-141-3p level was 1.17-fold less in early PCas than that in BPHs ($P = 0.2053$) (**Figure 1(b)**). This may be due to the limited number of cases analyzed. Taken together, these data provide evidence that miR-141-3p expression is not evidently changed in early PCa.

3.2. AR Is a Direct Target of miR-141-3p, and AR Levels Are Inversely Correlated with miR-141-3p Levels

AR overexpression is well known to play an important role in the pathogenesis of PCa. We investigated whether certain miRNA(s) contribute to AR upregulation. From RNAhybrid 2.0 [11], we predicted that the region spanning 2563 -

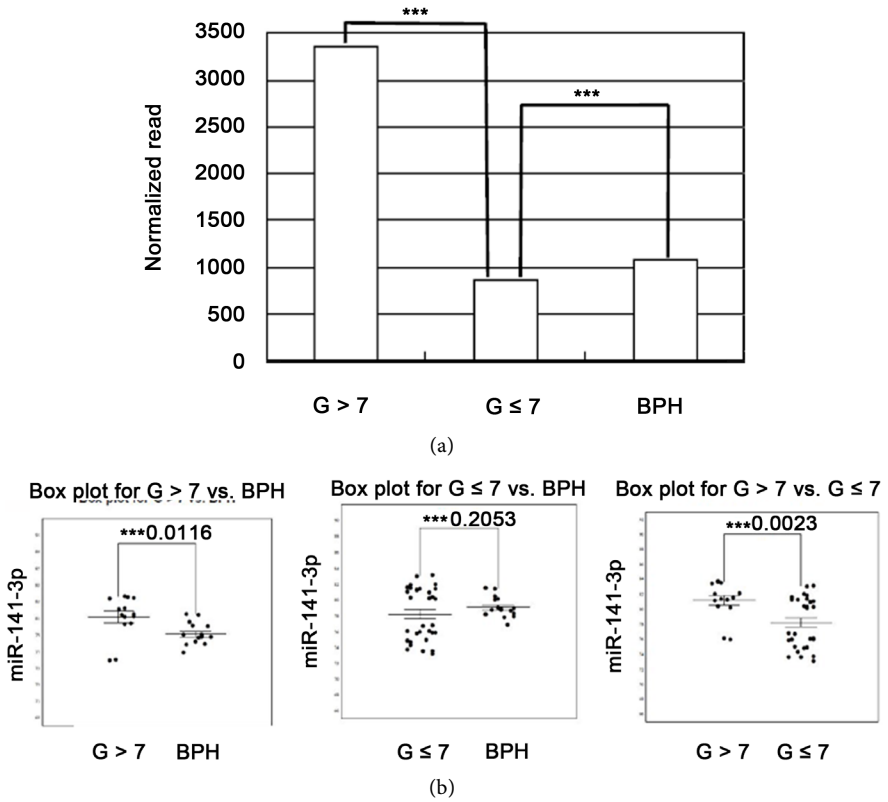

Figure 1. MiR-141-3p expression in human prostate cancer and benign prostatic hyperplasia (BPH). MiR-141-3p expression levels in tissues were defined by deep-sequencing (a) and qRT-PCR (b). The average expression difference between early tumors and noncancerous tissues was deceased by 1.26-fold (a) and 1.17-fold (b), while the expression difference between malignant and early tumors was increased by 6.7-fold (A) and 1.5-fold (b). MiR-141-3p expression levels were not evidently changed in early prostate tumor tissues compared with those the BPH tissues. (a) MiR-141-3p expression levels were assessed by deep-sequencing in noncancerous tissues (n = 9), early tumors (G ≤ 7, n = 8), and malignant tumors (G > 7, n = 7); (b) MiR-141-3p expression levels were analyzed in 32 FFPE tissues of early PCa (G ≤ 7), 14 malignant PCa tissues (G > 7) and 16 BPH tissues using qRT–PCR. U6 small nuclear RNA was used as an internal control. Horizontal lines indicate the median. A Student's *t*-test was used to analyze significant differences among the groups. (***) Significant difference when compared with control tissues ($P < 0.05$).

2585 bp of the AR mRNA (GenBank: M23263.1) was a potential miR-141-3p binding site (**Figure 2(a)**). Sequence alignment of this putative site showed evolutionary conservation within mammalian species and AR mutants (**Table 1**).

To verify that the putative miR-141-3p binding site in the ORF of AR mRNA is responsible for regulation by miR-141-3p, the region spanning 2550 - 3000 bp of the AR ORF (5'-GCCATTGAGCCAGGTGTAGTGTG-3') and its mutant (5'-GCCA-------AG-3') were cloned separately into the pmirGLO luciferase reporter vector, and then cotransfected with the miR-141-3p mimic or control miR-NC into 293T cells. Luciferase activity was measured after two days. As shown in **Figure 2(b)**, the miR-141-3p mimic significantly reduced luciferase levels when cotransfected with the wild-type AR ORF (8.9% inhibition, $P <$

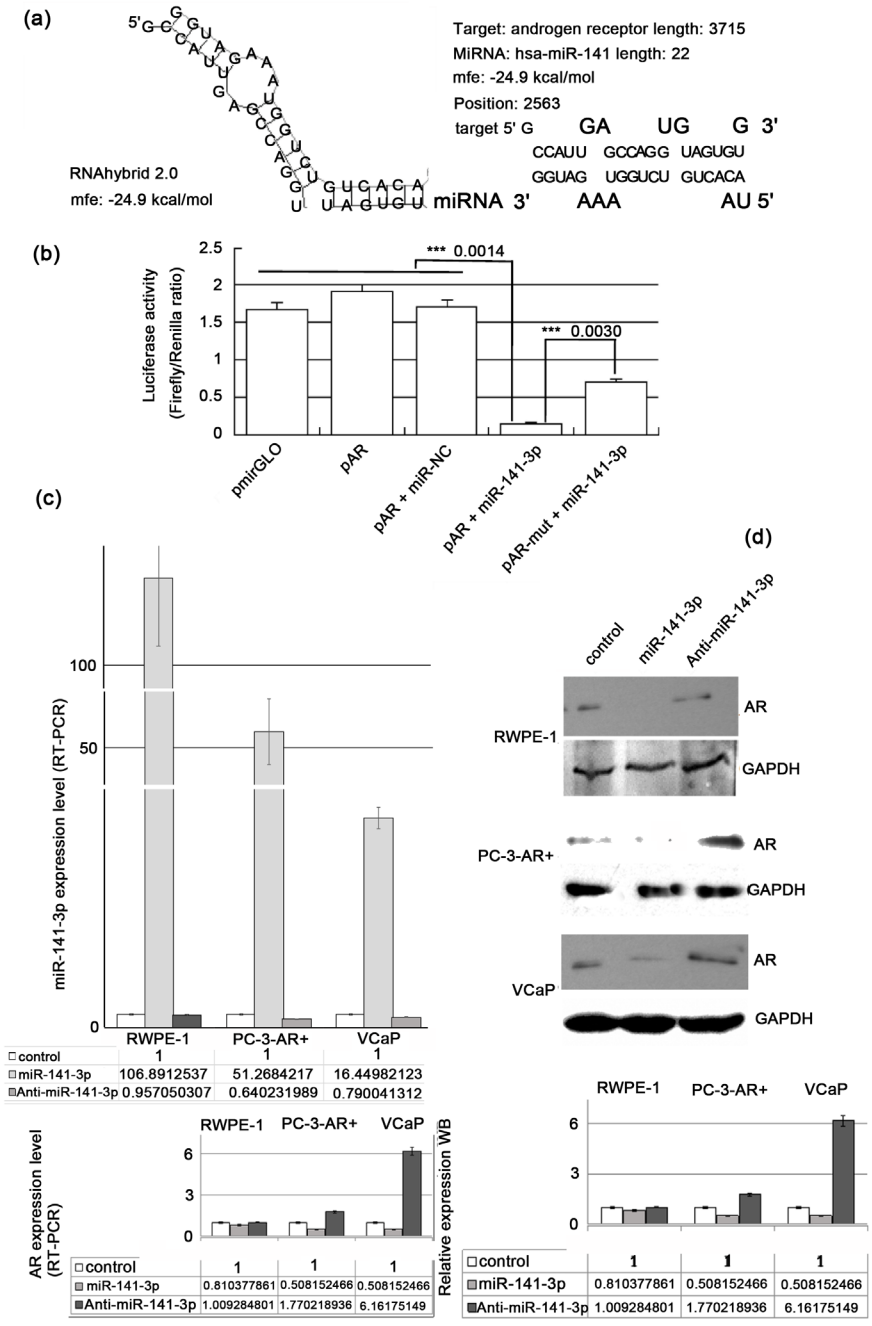

Figure 2. AR is a direct target of miR-141-3p, and AR levels are inversely correlated with miR-141-3p levels in RWPE-1 cells. (a) The region spanning 2563 - 2585 bp of the AR ORF (GenBank: M23263.1) was predicted as a potential miR-141-3p-binding site by RNAhybrid v2.0 software; (b) Relative luciferase activities were obtained by cotransfection of the miR-141-3p mimic or control miR-NC and pmirGLO reporter plasmids including AR-WT and AR-Mut, and calculated as the ratio of firefly/Renilla activities and normalized to those of the control; (c) MiR-141-3p and AR expression levels in RWPE-1, PC-3 and VCaP cells were determined by qRT-PCR analyses. Data represent the mean ± SD of three independent experiments; (d) The expression levels of AR in transfected RWPE-1. PC-3 and VCaP cells were detected by WB, and fold changes were calculated as the ratio of AR and GAPDH levels. Overexpression of miR-141-3p inhibited AR expression at the mRNA and protein levels, while miR-141-3p knockdown resulted in recovery of AR expression at both the mRNA and protein levels.

Table 1. The conservation analysis of miR-141-3p binding site in the AR ORF.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Homo sapiens androgen receptor (AR), mRNA	46.1	46.1	100%	0.004	100%	NM_001011645.2
Homo sapiens isolate AR_758 mutant androgen receptor (AR) isoform 1, complete cds	46.1	46.1	100%	0.004	100%	HM010955.1
Homo sapiens isolate AR_579 androgen receptor (AR) isoform 1, complete cds	46.1	46.1	100%	0.004	100%	GU784859.1
Homo sapiens isolate AR_649 androgen receptor (AR) isoform 1, complete cds	46.1	46.1	100%	0.004	100%	GU784858.1
Homo sapiens isolate AR_142 androgen receptor (AR) isoform 1, complete cds	46.1	46.1	100%	0.004	100%	GU784857.1
Homo sapiens isolate AR_277 androgen receptor (AR) isoform 1, complete cds	46.1	46.1	100%	0.004	100%	GU784856.1
Homo sapiens isolate AR_395 androgen receptor (AR) isoform 1, complete cds	46.1	46.1	100%	0.004	100%	GU784855.1
Homo sapiens isolate AR_473 androgen receptor (AR) isoform 1, complete cds	46.1	46.1	100%	0.004	100%	GU373805.1
Homo sapiens androgen receptor variant 5,6,7es (AR) mRNA, alternatively spliced	46.1	46.1	100%	0.004	100%	GU208210.1
Androgen receptor [human, Genomic Mutant, 370 nt]	46.1	46.1	100%	0.004	100%	S79368.1
Nomascus leucogenys androgen receptor (AR), mRNA	46.1	46.1	100%	0.004	100%	XM_003272706.3
Pongo abelii androgen receptor (AR), mRNA	46.1	46.1	100%	0.004	100%	XM_009234939.1
Pan paniscus androgen receptor (AR), mRNA	46.1	46.1	100%	0.004	100%	XM_003816907.2
Ochotona princeps androgen receptor (AR), mRNA	46.1	46.1	100%	0.004	100%	XM_004595204.1
Crocuta crocuta androgen receptor mRNA	46.1	46.1	100%	0.004	100%	AY128705.1
Wallabia bicolor androgen receptor (AR) gene, exon 4 and partial cds	46.1	46.1	100%	0.004	100%	AF081532.1
Pan troglodytes androgen receptor (AR), mRNA	46.1	46.1	100%	0.004	100%	NM_001009012.1
Gorilla gorilla gorilla androgen receptor-like (LOC101143627), mRNA	40.1	40.1	86%	0.26	100%	XM_004064298.1
Sus scrofa androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_013986227.1
Ictidomys tridecemlineatus androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_005335195.2
Aotus nancymaae androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_012460838.1
Macaca nemestrina androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_011732842.1
Cercocebus atys androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_012060885.1
Colobus angolensis palliatus androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_011961988.1
Mandrillus leucophaeus androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_011980535.1
Rhinopithecus roxellana androgen receptor (LOC104668178), mRNA	38.2	38.2	100%	1.0	96%	XM_010370828.1
Papio anubis androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_003917817.2
Fukomys damarensis androgen receptor (AR) mRNA, partial cds	38.2	38.2	100%	1.0	96%	KF574039.1
Chlorocebus sabaeus androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_007991938.1

Continued

Lipotes vexillifer androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_007445570.1
Balaenoptera acutorostrata scammoni androgen receptor-like (LOC103002585), mRNA	38.2	38.2	100%	1.0	96%	XM_007185461.1
Physeter catodon androgen receptor-like (LOC102979424), mRNA	38.2	38.2	100%	1.0	96%	XM_007113425.1
Elephantulus edwardii androgen receptor-like (LOC102875920), mRNA	38.2	38.2	100%	1.0	96%	XM_006901989.1
Macaca fascicularis androgen receptor-like (LOC102117720), mRNA	38.2	38.2	100%	1.0	96%	XM_005593810.1
Condylura cristata androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	XM_004694437.1
Octodon degus androgen receptor (Ar), mRNA	38.2	38.2	100%	1.0	96%	XM_004644511.1
Trichechus manatus latirostris androgen receptor (LOC101351426), mRNA	38.2	38.2	100%	1.0	96%	XM_004380092.1
Pig DNA sequence from clone CH242-427P7 on chromosome X, complete sequence	38.2	38.2	100%	1.0	96%	CU469162.12
Callithrix jacchus androgen receptor mRNA, complete cds, alternatively spliced	38.2	38.2	100%	1.0	96%	GU126669.1
Oryctolagus cuniculus androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	NM_001195724.1
Saimiri boliviensis androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	NM_001279963.1
Macaca mulatta androgen receptor (AR), mRNA	38.2	38.2	100%	1.0	96%	NM_001032911.1
Papio hamadryas androgen receptor mRNA, complete cds	38.2	38.2	100%	1.0	96%	U94176.1

0.05), but not with the mutant AR ORF, indicating a direct interaction between miR-141-3p and AR mRNA.

To further confirm that miR-141-3p downregulates the levels of AR mRNA and protein, RWPE-1, PC-3 and VCaP cells were transfected with the miR-141-3p mimic or control miR-NC, and AR mRNA and protein levels were evaluated by qRT-PCR and WB analyses, respectively (**Figure 2(c)** and **Figure 2(d)**). Our data showed that miR-141-3p downregulated AR mRNA and protein levels. Similarly, miR-141-3p knockdown led to recovered AR expression in these cells (**Figure 2(c)** and **Figure 2(d)**). Taken together, these data indicate that miR-141-3p targets the AR and affects AR activity.

3.3. MiR-141-3p Inhibits Prostate Epithelial Cell Proliferation and Mobility

To analyze the role of miR-141-3p in early progression of human PCa, we upregulated or downregulated miR-141-3p to observe its influence on cell proliferation, cell cycle and mobility ability. We chose immortalized normal human prostatic epithelial cell line RWPE-1 cells to study the functions of miR-141-3p, because the genome and phenotype of these cells are similar to those of normal prostatic epithelial cells. In this cellular system, we were able to reproduce the development process of early PCa induced by miR-141-3p and AR. RWPE-1 cells were transfected with the miR-141-3p mimic or miR-NC, and analyzed for

cell growth, cell cycle and mobility. MiR-141-3p-transfected RWPE-1 cells expressed the miRNA at a level 106-fold higher than that of the control (Figure 2(c)). Proliferation assays showed that cell growth was reduced in miR-141-3p-transfected cells compared with miR-NC-transfected control cells (Figure 3(a)). In FCM analysis, the percentage of cells in the G0/G1 phase increased from 73.29% in controls to 75.75% in miR-141-3p-transfected cells, whereas the percentage of cells in S phase decreased from 18.70% to 16.20% (Figure 3(b)), indicating that miR-141-3p overexpression effectively inhibited the transition from G_0/G_1 to G2-S-M phase in the progression of human early PCa. We analyzed the effect of miR-141-3p overexpression on the mobility behavior of RWPE-1 cells. MiR-141-3p overexpression decreased the mobility of cells (Figure 4). In loss experiments of miR-141-3p, miR-141-3p knockdown led to enhanced cell growth and mobility as shown in Figure 3 and Figure 4.

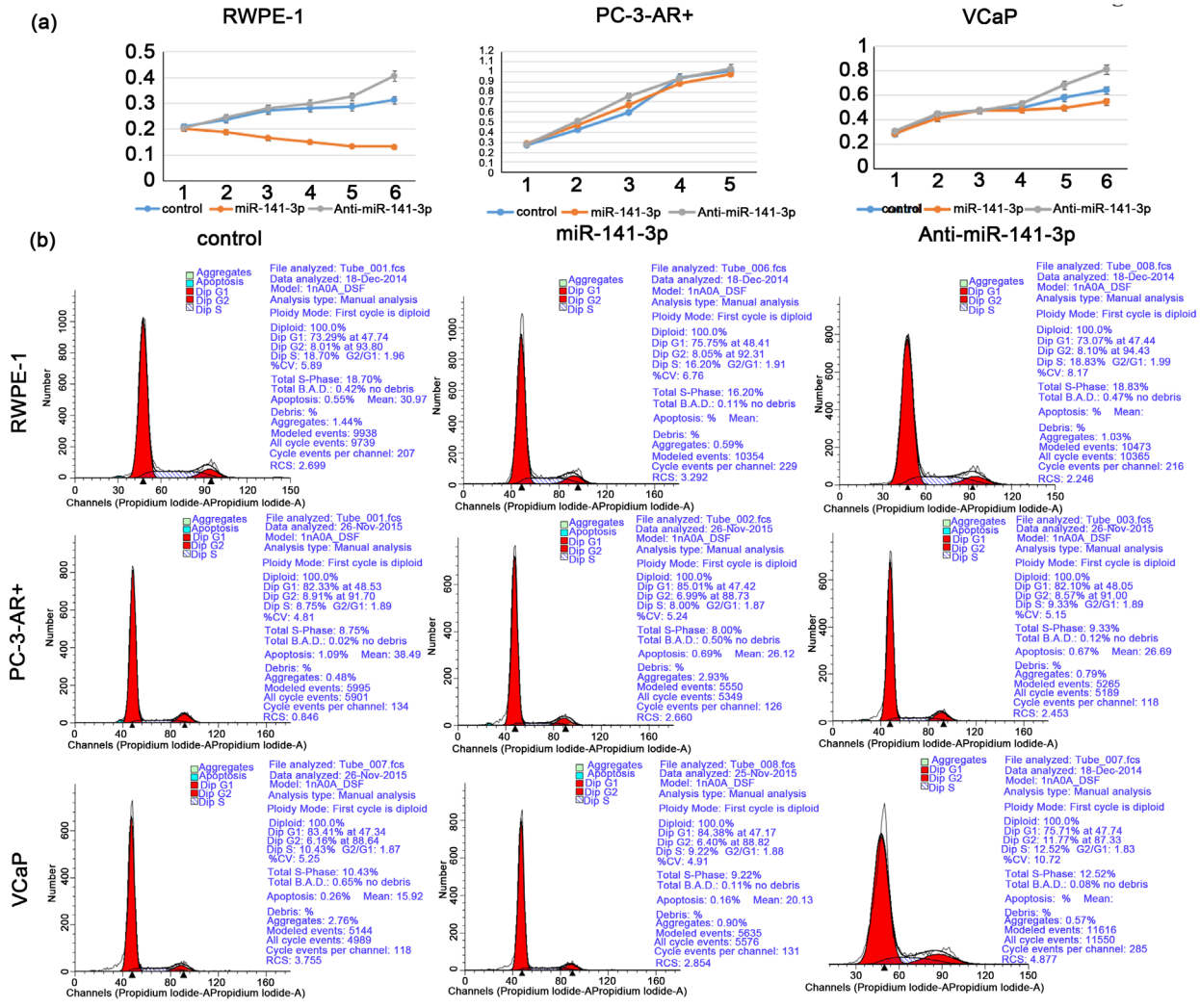

Figure 3. MiR-141-3p inhibits cell proliferation *in vitro*. (a) Cell growth curves were generated based on MTT assay data. Transfected cells were seeded at 3000 cells per well in a 96-well plate. Absorbance was measured at the indicated time points using a BioRad Model 680 microplate reader. Data represent the average of three independent experiments; (b) Cell cycle distribution of transfected cells with the miR-141-3p mimic or inhibitor or miR-NC. Overexpression of miR-141-3p hindered the G1 to G2-S-M cell cycle transition.

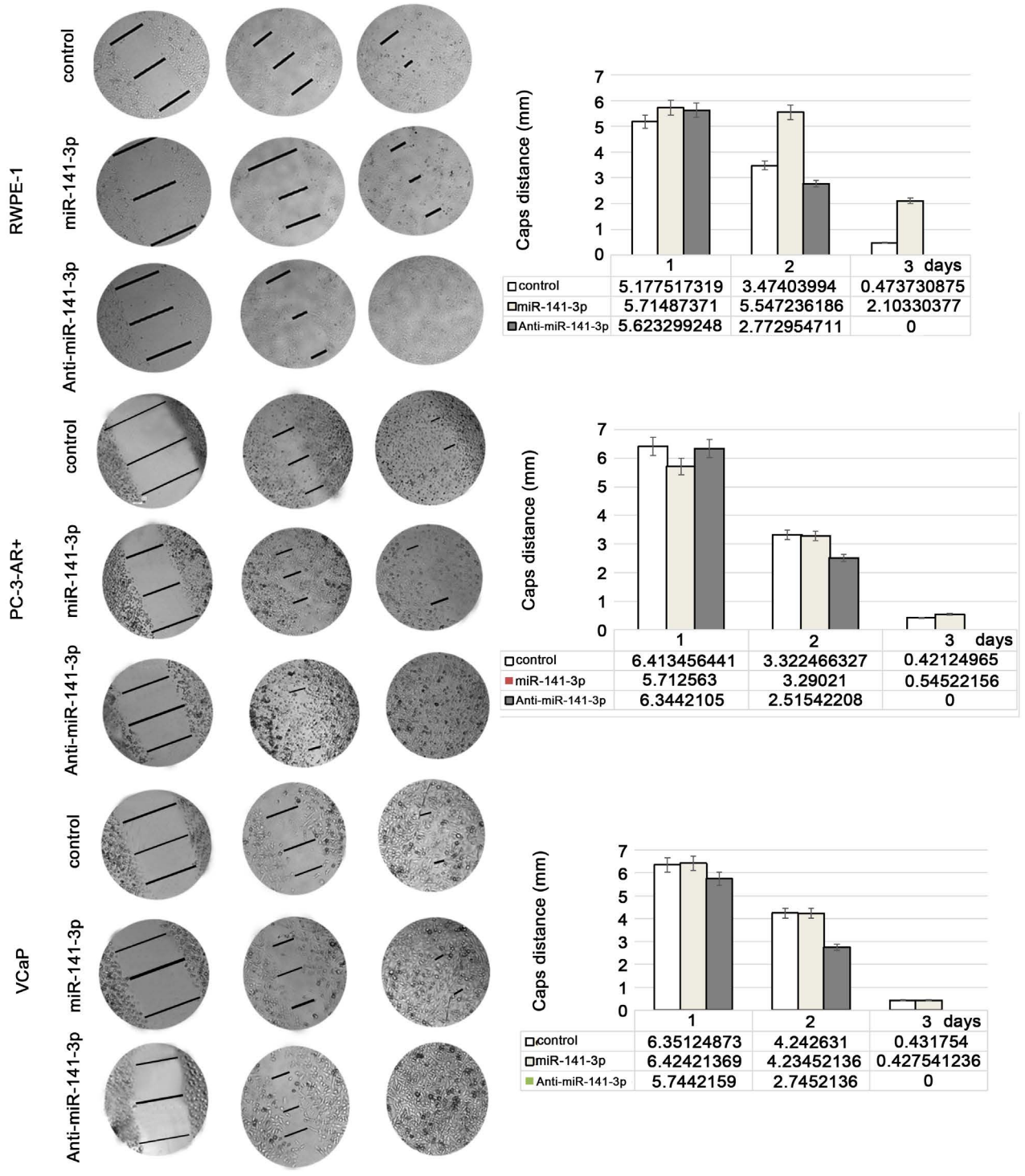

Figure 4. MiR-141-3p inhibits cell mobility *in vitro*. RWPE-1, PC-3 and VCaP cell wound-healing assays. The wound gaps were photographed and measured in three different areas. Wound-healing assays indicated that AR downregulation inhibited cell mobility.

To further evaluate the association of miR-141-3p with PCa progression, we chose two human malignant prostate cancer cell lines PC-3 and VCaP to assay their cell growth, cell cycle and mobility. As shown in Figure 3 and Figure 4, the same alteration was observed, but miR-141-3p's effection to cell functions in

PC-3 and VCaP cells was slightly compared to that in RWPE-1 cells.

4. Discussion

Although the prostate is dependent on androgens for growth and development, certain growth restriction mechanisms exist in the normal state to avert androgen-induced over-growth. It is imperative to reveal how AR mediates these actions and breaks the growth restriction mechanisms, leading to prostate carcinogenesis. Thus, in this study, we attempted to systematically identify miRNAs that bridge the AR pathways to exert the cellular phenotypic effect in PCa. To study how miRNAs regulate AR signaling, it is necessary to identify their targets. Seed-sequence-based predictions, such as TargetScan, miRanda, RNAhybrid and PicTar databases, provide necessary information to identify the actual miRNA targets. MiR-141-3p is androgen regulated in PCa cell lines and xenografts [12], and modulates transcriptional activity of AR by targeting the small heterodimer partner protein (Shp), a corepressor to AR [13]. Additionally, Wang *et al.* found that miR-141-3p regulated the expression of androgen receptor by targeting its 3'UTR in prostate cancer LNCaP cells [14]. In our study, we found that miR-141-3p bound specifically to the region spanning 256 - 2585 bp of the AR ORF to decrease the levels of AR mRNA and protein. Moreover, miR-141-3p is reported to be significantly overexpressed in serum from CRPC patients compared with serum from low-risk localized patients. In high-risk prostate tumor samples, miR-141 is also expressed at significantly higher levels compared with those in normal prostate tissues [4] [6], and elevated serum miR-141-3p levels are observed in patients with metastatic PCa [5] [10]; however, few studies describe the changes in miR-141-3p expression in the early stages of the disease. Hizir *et al.* [15] reported that miR-141-3p expression is at normal levels in early PCa, which is in accordance with our findings. Using deep-sequencing analysis, we found that miR-141-3p expression was decreased by 1.26-fold in early PCa compared to that in BPH ($P < 0.05$), and confirmed a similar 1.17-fold decrease in early PCa compared to that in BPH ($P = 0.2053$) by qRT-PCR. In addition, miR-141-3p expression levels vary in different tumors, with overexpression observed in bladder [16], urothelial [17], breast [18] [19], ovarian [20], endometrioid [21], cholangiocytes [22] and colorectal cancers [23] and NSCLC [24], while expression is downregulated in gastric cancer [25], hepatocellular carcinomas [26] [27], renal cell carcinoma [28], lymphatic metastatic pancreatic cancer [29], pituitary tumors [30], craniopharyngioma [31], head and neck cancer [32], osteosarcoma [33], and cutaneous T cell lymphoma [34]. These reports provide compelling evidence for a role of miR-141-3p as an oncogene or tumor suppressor in different tumors and at different stages.

The results of our biological experimental validation reveal several interesting and novel mechanisms that significantly contribute to PCa cell survival and pathogenesis. Our present study also revealed some possible mechanisms by which androgen growth restriction is disrupted. Although we focus on AR in this study, we realize that miR-141-3p targets hundreds of human genes (**Table 2**),

Table 2. Validated target genes of miR-141-3p.

MicTarBase 4.5	TarBase 7.0
ACVR2B, BAP1, BRD3, CDYL, CLOCK, CTBP2, DLX5, E2F3, EIF4E, ELAVL4, ELMO2, ERBB2IP, HMGB1, HOXB5, KLF5, KLF11, KLHL20, MAP2K4, MAPK9, MAPK14, NR0B2, PPARA, PTEN, PTPRD, RASSF2, RIN2, SEPT7, SERBP1, SFPQ, SHC1, SIP1, STK3, TCF7L1, TFDP2, TGFB2, TRAPPC2P1, UBAP1, VAC14, WDR37, YWHAG, ZEB1, ZEB2, ZFPM2,	BAP1, BRD3, CLOCK, CyclinD1, DP2, E2F3, ELMO2, ERBB2IP, HOXB5, HuD, JNK2, KLF5, KLF11, KLHL20, MAP4K4, PTEN, PTPRD, RASSF2, RIN2, SEPT7, SFPQ, SHC1, STK3, TCF7L1, TGFB2, UBAP1, VAC14, WDR37, YWHAG, ZEB1, ZEB2

Lineation emphasized target genes which are recorded by two databases.

and other targets may also be relevant in the tumor suppressive function of miR-141-3p in PCa. Although AR was the predicted as a direct target of miR-141-3p according to our data, PTEN and TCF7L1, which are involved in G1/S progression, and E2F3, which is involved in cell proliferation, have also been identified as miR-141-3p targets, and may also be involved in the PCa signaling pathways.

5. Conclusion

In this study, we show that miR-141-3p expression does not change in early PCas. MiR-141-3p negatively regulates AR expression, and miR-141-3p down regulation increases expression of AR. Therefore, we propose that deregulation of miR-141-3p contributes to the high levels of AR expression in clinical PCas. Additionally, our data suggest that miR-141-3p down regulation contributes to the pathogenesis of PCa.

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Conflict of Interest

The authors disclose no potential conflicts of interest.

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Supplementary

Table S1. Clinical characteristics of patient set 1.

	Group 1	Group 2	Group 3
Gleason	G > 7	G ≤ 7	Non-cancer
Samples	7 (29.17%)	8 (33.33%)	9 (37.5%)
Age			
Median	67	70	72
Mean	69.43	70.25	72.25
SD	6.73	3.15	8.62
Min - Max	60 - 79	66 - 76	62 - 83

Table S2. Clinical characteristics of patient set 2.

	Group 1	Group 2	Group 3
Gleason	G > 7	G ≤ 7	Non-cancer
Samples	14 (22.58%)	32 (51.61%)	16 (25.81%)
Age			
Median	65	68	68
Mean	67.25	66.94	68
SD	7.42	7.87	4.54
Min - Max	56 - 77	52 - 82	62 - 76

Table S3. Primer of qRT-PCR.

	RT primer	F primer	R primer	Tm
AR	Oligo(dT)18	5'-cctgctcaagacgcttctac-3'	5'-ttccaatgcttcactgggtg-3'	61.7
GAPDH	Oligo(dT)18	5'-cggagtcaacggatttggtcgtat-3'	5'-agccttctccatggtggtgaagac-3'	72.8
miR-141-3p	5'-gtcgtatccagtgcagggtccgaggtattcgcactggatacgacccatct-3'	5'-cgagcgtgtaacactgtctggtaa-3'	5'-cagtgcagggtccgaggtatt-3'	68.2
U6	5'-cgcttcacgaatttgcgtgtca-3'	5'-gcttcggcagcacatatactaaaat-3'	5'-cgcttcacgaatttgcgtgtca-3'	69.4

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Association between Cancer and Environmental Exposure to Glyphosate

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Abstract

Background: Argentina, Brazil, Paraguay and Uruguay farm transgenic seeds glyphosate resistant. Argentina annually utilizes 240,000 tonnes of glyphosate in agriculture. A change in the profile of morbidity and mortality is perceived in agricultural areas; cancer seems to prevail. Monte Maíz is a typical argentine agricultural town with 8000 inhabitants; the Mayor and residents of Monte Maiz requested an environmental health study due to perceived increase in cancer frequencies. **Methods:** An exploratory ecological study was developed to assess the urban environmental contamination and the frequencies and distribution of cancer through an environmental analysis of pollution sources including measurements of pesticides in water, soil and grain dust, and a cross-sectional study of cancer patients that explore associations with different variables. **Results:** Glyphosate was detected in soil and grain dust and was found to be at an even higher concentration in the village soil than in the rural area. 650 tonnes are used annually in the region and manipulated inner town. We do not find other relevant sources of pollution. Cancer incidence, prevalence, and mortality are between two and three times higher than the reference values (Globocan 2012, WHO) for the entire nation (706/100,000 persons vs. 217/100,000; 2123/100,000 persons vs. 883.82/100,000 and 383/100,000 persons vs. 115.13/100,000, respectively). **Conclusion:** This study detects high glyphosate pollution in association with increased frequencies of cancer in a typical argentine agricultural village, and by design, cannot make claims of causality. Other study designs are required, but if we corroborate the concrescence of high exposure to glyphosate and cancer.

Keywords

Glyphosate, Pesticides, Cancer, Environmental Health, Environmental Exposure

1. Background

In 1996, Argentina began to grow genetically modified (GM) seeds, and used currently 25 million hectares where 12 million people live; these crops have generated a substantial increase in the pesticide consumption. In 2013, Argentina sprayed 240,000 tons of Glyphosate [1] [2]. A change in the profile of morbidity and mortality is perceived for physicians of agricultural areas; now cancer seems to prevail [3]. Epidemiological and experimental researchers suggest a positive association between glyphosate and cancer, as recently reported by the International Agency for Research on Cancer (IARC)-World Health Organization [4].

The town Monte Maiz (Union District in Province of Cordoba) lies at the heart of Argentina's agricultural area, a region of greater agricultural productivity in the country, where soy, maize, and wheat are grown, and is in the center of the country. In recent years, local governmental authorities along with local residents and doctors were worried about an increase apparent in the number of people suffering from diseases like cancer and thus requested an assessment of health status to the Faculty of Medical Sciences, National University of Cordoba (UNC). The community had conducted a health census in 2007 (unpublished), carried out by teachers and other volunteers, in which high rates of cancer were identified. Also, the Provincial Tumors Registry of the Province of Cordoba (RPT) reported that Union District has a higher cancer mortality rate than that recorded in Cordoba City [5]; nevertheless, there are few epidemiological studies on the environmental health of rural populations in Argentina and very little has been published to date.

The objective of this study of the Monte Maíz environmental contamination recorded mainly, the presence of glyphosate and other pesticides and checked whether the incidence, prevalence, and mortality of cancer were increased. It was our goal to verify concurrence of glyphosate exposure and cancer.

2. Material and Methods

An exploratory ecological study on cancer and environmental pollution was performed. Consisting on the one hand of an epidemiological study (a cross-sectional study) with a household survey of health orientation of the whole population (population survey), designed to geo-reference each record in the village with use of nine ratios census (R) by National Institute Census that divide the town into nine sectors outweighed demographically as seen in the map of **Figure 1**. Through the household survey, we checked cancer prevalence (living residents diagnosed in the last 5 years with oncological disease in any location), cancer incidence 2014 (new cases diagnosed last year) and cancer mortality (deaths

Figure 1. Map of the Census Radius of Monte Maíz by National Institute Census divides the town into nine sectors outweighed demographically, from in number 9 to number 18.

from cancer in the last year and the past 5 years), for criteria of Globocan 2012 [6], these three were dependent variables, while sex, age, occupation, stay in the village, smoke, ratio census residence, educational level and the presence of environmental contaminants were the independent variables.

On the other hand, an environmental analysis recording sources of contamination such as landfills, cell site (cell tower), electric power transformers, industrial sites, stockpiles of grains, storehouses of pesticides, and spraying machines. We interviewed community and government stakeholders, business owners, city officials, teachers, farmers and workers that sprayed pesticides, in order to recognize the performance of industries, local public services, and agribusiness (drinking water, sewer management, household waste, industries pollutions, routines and doses of pesticides use).

Samples of environmental matrices (water, soil, grain husks) were collected and analyzed by the Center for Environmental Research, Faculty of Exact Sciences of National University of La Plata, which selected twelve internal and peripheral sites in the town to examine the presence of glyphosate, its metabolite aminomethylphosphonic acid (AMPA), and currently used pesticides (chlorpyrifos, endosulfan, atrazine, 2.4D, and epoxiconazole). Both pretreatment and analysis of pesticides were performed under international regulations using liquid chromatography-mass spectrometry [7] [8]. A dosage of arsenic (As) was conducted in domestic water network using hydride generation atomic absorption spectrometry.

The study area was Monte Maíz, a town located on Provincial Route N˚11', 33˚12' South latitude and 62˚36' West longitude of Greenwich, at a height above

sea level of 114 metres; the town is 113 years old and has 7788 inhabitants (8045 including residents of surrounding rural areas). Agriculture is the main economic activity with complementary metalworking industry that is located on the Southern edge of the town [9].

2.1. Statistical Analysis

Crude rates were obtained through a database and numerical matrix. The incidence rate was age adjusted to the structure of the population of Cordoba city by the indirect method. A bivariate correlation Pearson analysis conducted to assess the association of cancer with independent variables, included the spatial distribution according to ratios census in which the town was divided (R09-R18). We built maps for cancer and pollution sources using Quantum GIS 2.4 software and created contingency tables to perform relational measurements between exposure and disease. For this end, the following software was used: INFOSTAT (UNC), SPSS, and EPIDAT (PAHO). Rates of cancer incidence, cancer prevalence and cancer mortality of Monte Maíz were compared with the same rates for Cordoba city (reference big city in the province), for the whole province and for the entire country, according to RPT [5], the National Health Ministry [10] and Globocan 2012 [6].

2.2. Study Conduct

Physicians or medical students carried out the fieldwork during October 2014; all health surveys were conducted by final year medicine students of UNC and medical professors. The study was conducted in accordance with the Declaration of Helsinki and under the framework of Act 9694 Article 2 of the Province of Cordoba in accordance with the law regulating health research and was approved by the Bioethics Committee established by this law for observational studies [11]. All surveys were performed after obtaining informed consent.

3. Results

3.1. Environmental Analysis

In Monte Maíz the electrical network is powered by medium-voltage power distributed in the urban area, with substations of 33 kV to 380 w, no high voltage. Has a sewer system with a domestic collection network reaching every home; solid urban waste is collected by a municipal service that has a Solid Waste plant. Their hinterland has soybeans on 45,000 ha and maize on 20,000 ha which are main summer crops and wheat on 15,000 ha as a winter crop. We identified an open landfill, 800 meters northeast of the town's limit, with no evidence of fire in the last 5 years. There is an absence of forestry across the periphery of the town, which is replaced by soybean and maize crops, starting at the immediate edge of houses. These crops are frequently treated, with pesticide, by ground equipment and crop dusters. At the southwest of Monte Maíz, we found two livestock breeding farms, and, on the west side, a flood zone, with ponds, a park, and a sewage treatment plant between the crop fields.

There are two farm equipment industries, located at the southern tip of the town; these factories use methane gas as a source of energy. The sources of electromagnetic radiation were two cellphone towers, located in R9 and R12 which are highlighted on the map of pollution factors on Monte Maíz in Figure 2 (there are two other towers located outside of the urban area). The population of Monte Maíz receives drinking water of very good quality, drinkable and arsenic-free. Within the inhabited village, there are silos of cereal from where soy and corn husks are released (grain dust) shown on the map in Figure 2 and were identified twenty-two deposits for spraying machines and pesticide containers used in the region.

Local agronomists and agrochemical applicators report that in Monte Maíz GM soybean and corn crops use 10 kilograms of glyphosate per ha per year. Six hundred and fifty tonnes of glyphosate are aerosolized in the area, creating a general burden of environmental exposure to glyphosate of 79 kg per person per year, which varies for agricultural or non-agricultural activity and for spatial distribution of glyphosate. This region utilizes 975 t of all pesticides each year.

Chemical contaminants test: Herbicide glyphosate and AMPA was detected in 100% samples of soil and husk. In grain husks from silos, glyphosate and AMPA prevailed (505 and 607 ppb), followed by chlorpyrifos (14 ppb) and epoxiconazole (2.3 ppb) as shown in Table 1. Sampling site N˚6 (map in Figure 2), belonging to a children's playground, contained 68 times more glyphosate than site N˚5, belonging to a farm field of corn resistant to glyphosate. Similarly, site Nº8, where the soil sample was taken from the sidewalk next to pesticides deposits, had the highest concentration of glyphosate (3868 ppb), AMPA (3192 ppb), and other pesticides.

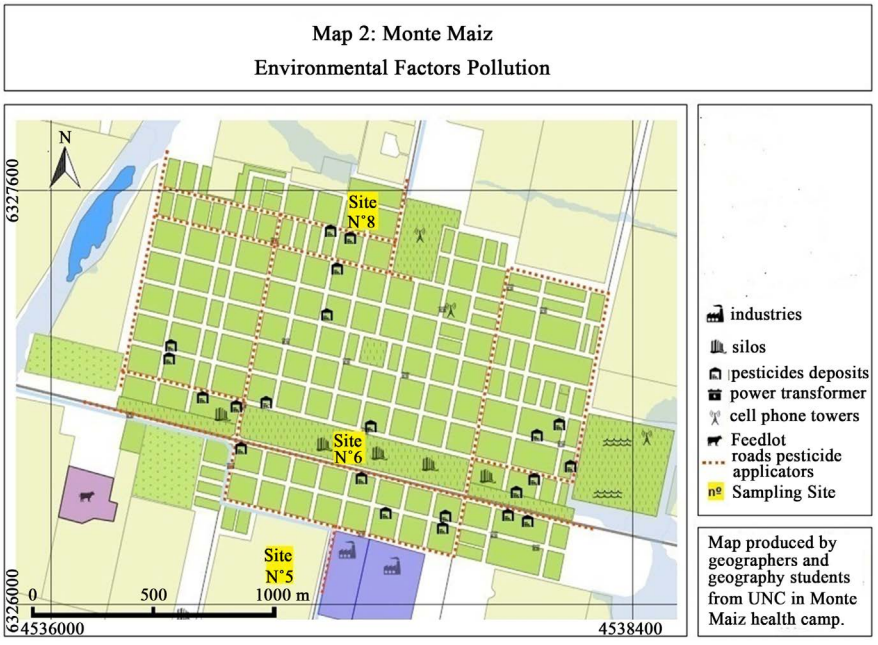

Figure 2. Map of environmental factors of pollution on Monte Maíz and main sampling site.

Table 1. Measurements pesticides in environmental matrices, main findings. Reference S located sampling site (S5, S6 and S8) in map of **Figure 2**. DNC: Detectable no quantifiable. Center for Environmental Research, Faculty of Exact Sciences of National University of La Plata.

S: Site sampling	Glifosato	AMPA	2.4 D	Atrazina	Clorpirifos	Endosulfan I	Endosulfan II	Cipermetrina	Epoxiconazol
S1 drinking water network	<2 ppb	<2 ppb	<1 ppb	<0.5 ppb	DNC	DNC	DNC	<0.005 ppb	<0.005 ppb
S5 crop field soil	41 ppb	116 ppb	<5 ppb	6.4 ppb	242 ppb	<1.5 ppb	2.2 ppb	58 ppb	3 ppb
S6 children's playground soil	2792 ppb	797 ppb	S/D	S/D	4.4 ppb	<1.5 ppb	<1.5 ppb	4 ppb	3.4 ppb
S6 children' playground grain husks from silos	505 ppb	607 ppb	S/D	S/D	14 ppb	DNC	<1.5 ppb	DNC	2.3 ppb
S8 pesticides deposits soil	3868 ppb	3192 ppb	128 ppb	52.5 ppb	150.4 ppb	17.5 ppb	338 ppb	180 ppb	6.3 ppb

Glyphosate also had the highest concentrations among all the matrices studied (3868 ppb), exceeding by far the other pesticides: endosulfan II (337.7 ppb) and chlorpyrifos (242 ppb) (see **Table 1**). There were minimal concentrations of pesticides in drinking water, also, the arsenic in drinking water was less than 5 ppb.

3.2. Epidemiological Analysis

Overall, 92% households were visited, 4.8% corresponds to households that refused to answer the survey. Some houses were uninhabited at the time of the visit. The information was collected from 4859 people (62% of the population), its characteristics are available in **Table 2**.

The crude cancer incidence rate 2014 was 706/100,000 persons (n: 35/4954), and age-adjusted rates (indirect method) was 980/100,000 (CI: 655-1305) for Monte Maíz, in Cordoba city (used as reference population) this rate was 469/100,000 confidence interval (CI): 453-484 and. The crude prevalence rate was 2123/100,000 persons (n: 104/4898).

The most common locations of cancer was found in breast 29% (n: 30/104), colon 10% (n; 11/104), prostate 8% (n: 9/104), thyroid 8% (n: 8/104), and skin 7% (n: 7/104). In relation to the age of patients, 22% of patients with cancer of Monte Maíz have less than 44 years old age.

The 2014 cancer mortality rate was 383/100,000 persons. Between 2010 and 2014, there were 68 confirmed cancer deaths (rate: 274/100,000). According to death certificates in 2013 and 2014, cancer deaths made up 39% and 34% of all deaths (**Table 3**).

Pearson correlation showed a link between cancer prevalence and R15 (people living in census radius n˚15) and cancer incidence with groups who engage in agricultural activities. Odds ratio (OR) for the prevalence of cancer in R15 was 2.15 (CI: 1.35 - 3.42) $p = 0.0009$, and incidence of cancer in agricultural families was 3.5 (CI: 1.45 - 8.58) $p = 0.002$. Smoking showed no relationship with cancer incidence or prevalence in Monte Maíz.

Table 2. Monte Maíz population surveyed: characteristics, absolute numbers and percentages

Characteristics	Magnitude
Number of individuals recorded	4959 (61.98% of population total)
Male	2361 (47.61%)
Female	2597 (52.37%)
Average age of Population	36.03 years
People 0 - 15 years	1175 (23.69%)
People ≥16 years with complete primary education	3313/3744 (88.49%)
People with health insurance	3859 (78.01%)
Percentage of people with ≥5 years residence time	4141/4328 (95.68%)
Families of rural workers, farmers and agronomists	270 families (970 people)
Smokers	793/3780 (20.98%)

Table 3. Cancer data summary and its comparison to reference data.

	Monte Maíz	Reference
Crude Cancer Incidence Rate 2014	706/100,000	259.4/100,000 Cordoba City*
Crude Cancer Incidence Rate 2014	706/100,000	217/100,000 Argentina 2012**
Indirect Adjustment Cancer Incidence Rate	980/100,000 (CI: 655 - 305)	469/100,000 Cordoba City
New cases per year (incidence)	35 cases/year	13.9 cases/year Cordoba (RPT)*
Cancer Prevalence Rate	2123/100,000	884/100,000 Argentina 2012**
Cancer patients < 44 years (percent)	22%	11%*
Relative Risk of cancer < 44 years	1.88 (CI: 1.31 - 2.70)	1 Province of Cordoba
Cancer Mortality Rate/100,000	383 year 2014	128 Cordoba City Year 2009*
Average Cancer Mortality Rate 5 years	274/100 000	135 Cordoba City*
Cancer Mortality 2013 (percent)	38.7% (2013)	20% Córdoba City (2010)***
Cancer Mortality 2014 (percent)	33.9% (2014)	20% Cordoba City (2010)***

*Data source: Provincial Tumors Registry of the Province of Cordoba (RPT); **Data source: Globocan 2012, International Agency for Research on Cancer (IARC)-World Health Organization; ***Data source: Estadísticas vitales. Ministerio de Salud. Provincia de Córdoba

4. Discussion

Seeds that are genetically manipulated to contain a transgene have the ability to survive in saturated environments with glyphosate, an herbicide used to eradicate other plants. Glyphosate interferes with the vital metabolism of plants, but not with transgenic plants for which an alternative metabolic pathway was generated through bioengineering. Since 1996, when GM soy was introduced in Argentina, its use has continued to expand due to the high profit generated by its commercialization and easy harvest [12], as the extension of this crop increases, so does the use of glyphosate. Currently, Argentina is using 240,000 tonnes of glyphosate per year. This has increased year-on-year as a consequence of herbi-

cide-resistant weeds requiring higher doses of glyphosate and the combined use of other herbicides as 2.4D, atrazine, etc. [13]. This increase has resulted in 5 kg of glyphosate per person per year as potential exposure burden for all inhabitants of the country, greater in agricultural areas.

Monte Maíz shows the effects of this agricultural model, as is a production boom in the region, a high standard of living among its population, and the relocation of local farmers in the village; these farmers left rural areas and moved with their families work equipment and supplies. Deposits of agricultural equipment are multiplying inside the village (twenty-two in total), the largest deposits in town are in R15, and are five pesticide storage sites. A total of 650 tonnes of glyphosate per year is concentrated, manipulated and has surrounded the town, which now faces fields that are sprayed daily. Glyphosate was found in 100% of soil and husk dust samples. The concentration was 10 times higher than that of other pesticides. This demonstrates that, of all pesticides that pollute the environment, glyphosate is the most prevalent. Concentrations found in inner town are several times higher than in the soil in cultivated fields (see **Table 1**), reaffirming the impression that the town is at the operational center of the sprayed area. Glyphosate is also high in grain dust, it is also accompanied by other pesticides which rule their presence inside the village due to their use in gardening.

In metalwork factories, no significant pollution was found. The density per km^2 of the source of electromagnetic radiation such as cell site, high voltage power lines, and electrical voltage transformers is low compared with source electromagnetic radiation density in big cities, which minimizes the value of this pollution. Nueva Cordoba, a neighborhood of Cordoba city, which is located on the same surface as Monte Maíz with a larger population, has nine cell sites, while there are only two towers in Monte Maíz [14], although, a weakness of the study is the lack of electromagnetic radiation direct measurements.

Moreover, household garbage management, sewage, and contaminant-free water (for 16 years now) remove these contaminating factors from the observed pathologies. Thus, pollution with glyphosate and to a lesser extent with other pesticides is the predominant factor in the environmental contamination analysis of Monte Maíz.

The Monte Maíz 2014 crude cancer incidence rate is 276% higher than Cordoba city 2009 for RPT in last published data [5]; GLOBOCAN 2012 [6] and Health Ministry [10] estimated for Argentina an incidence 317% lower than in Monte Maíz (**Table 3**). RPT estimates 9,000 new cancer cases per year for the entire province; in the observed population, there should be 13.9 new cases in 2014, for IARC's references, the annual figure should not exceed 11 cases, however there are 35 cases in 2014. RPT data is generated from oncologist and pathologist reports and state statistics offices; there may be underreporting of cases. By contrast, our data may be biased due to the fact that they are self-referenced, and while this is a limiting factor for any study of disease through surveys, it is unlikely in some less prevalent pathology where, on the contrary, the most common error is type II. The biases that arise when comparing different

populations such as a farming town (Monte Maíz) and a large city (Cordoba City) refer to a greater relative weight of elderly people in farming towns that require adjustment rates by age; they do not substantially alter the differences in incidence found. The Monte Maíz adjusted incidence of cancer rate resulted in being 208% greater than in Cordoba city. As a secondary analysis but in view of the age bias, we also contrasted the age structure of the cancer cases reported by the RPT in years 2004-2009 with Monte Maíz age structure cancer cases data 2010-2014, compared between patients under 44 and over 45 years old age, and found that 22% of the patients in Monte Maíz are younger than 44 years old and 11% in the entire province. The OR for cancer in people younger than 44 in Monte Maiz was 1.88 (CI: 1.31 - 2.70) p-value: 0.001. It was observed that cancer appears in younger people in Monte Maíz; the findings are consistent with the observations made by local doctors in two ways: absolute increase of cancer and a greater relative presence of young cancer patients.

Cancer prevalence is 240% higher than what GLOBOCAN 2012 (IARC 2012) report for Argentina (**Table 3**). The urban area (radius census) with most important pesticide deposits, R15, showed a higher rate of cancer prevalence compared to all other radius into which the town was divided, suggesting a relation dose (exposure)-effect that would strengthen the inference of the relationship. Smoking did not influence and cancer locations do not differ from those reported by the RPT for the entire Province [5]. Simultaneously (March 2015), an environmental health analysis conducted for National University of Rosario (UNR) in another farming town (Maria Juana), located 300 km from Monte Maíz, detected 80 cancer patients among 3940 inhabitants (unpublished data) with a prevalence rate of 2013/100,000, very similar to our results. The UNR also analyzed the environmental health status of 19 towns in the agricultural region and found an increase of 2 - 4 times the expected cancer prevalence [15].

The Monte Maíz cancer mortality rate was 299% higher than the Cordoba city. From RPT, the average cancer mortality rate in the Union District is also twice as high as in Cordoba city [5]. From Health Ministry 20% of deaths in Argentina were due to cancer [16], it is also 20% cancer mortality in Cordoba city. However, according to death certificates in Monte Maíz, cancer deaths were 39% in 2013 and 34% in 2014. Serrano published in 2013 a study of cancer mortality in San Vicente, an agricultural town 290 km of Monte Maíz, where cancer multiplied in recent years together with the expansion of soybean cultivation and use of pesticides [17]. A multi-center study sponsored by the Health Ministry in 2012 reports substantial mortality differences between soybean farming villages (that used glyphosate) and cattle-raising villages (that do not use glyphosate), in Avia Terai, Campo Largo, and Napenay village, there were cancer deaths with frequencies of 31.3%, 29.8% and 38.9%, respectively, whereas in Cole-Lai and Charadai, only 5.4% and 3.1% [18]. Another study as well, sponsored by the Health Ministry on pesticide exposure and health found a connection between male cancer mortality and breast cancer mortality distribution with glyphosate use rate for districts [19]. During 2015 the UNR School of Medicine studied the

environmental health of the agricultural town San Salvador in Entre Rios, found that almost 50% of those who died did so for cancer in recent years and high contamination with pesticides, including glyphosate [20].

Cancer and agrochemicals is a mentioned relationship in epidemiological and experimental reports. Leu and Swanson found a strong link between health deterioration and increased cancer rates with glyphosate exposure in the USA [21]. The Monograph Working Group of the IARC in Evaluation of Carcinogenic Risks to Humans in 2015 reviews 1000 studies on glyphosate and chooses 200 relevant paper to conclude that "There is limited evidence in humans for the carcinogenicity of glyphosate. A positive association has been observed for non-Hodgkin lymphoma. Also, there is sufficient evidence in experimental animals for the carcinogenicity of glyphosate. Concluding that Glyphosate is probably carcinogenic to humans (Group 2A). There is strong evidence that glyphosate can operate through two key characteristics of known human carcinogens, and that these can be operative in humans. Specifically: there is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans *in vitro* and studies in experimental animals; And there is strong evidence that glyphosate, glyphosate-based formulations, and AMPA can act to induce oxidative stress based on studies in experimental animals, and in in-vitro studies in humans" [4].

Studies of glyphosate genotoxicity emphasize the occurrence of damage to the DNA strands that when not repaired nor the cell removed, can lead to cell mutations that are the start of biological onset of cancer [22] [23] [24] [25]. So far, epidemiological and experimental evidence shows that structural and numerical chromosomal aberrations (CAs) generated by genotoxic agents are involved in carcinogenesis [26]. Near Monte Maíz, in Marcos Juarez City, two studies showed twice the frequency of CAs in environmentally exposed people to glyphosate or other pesticides [27] and genotoxicity in children exposed to pesticides comparing to not expose [28].

Our epidemiological link between environmental glyphosate and cancer seems consistent regarding the incidence, prevalence, and mortality, the strength of the association appear important and highlights the fact that families with farming activities have a greater risk of cancer than families no farming, probably due to greater direct exposure to glyphosate; although recognize that the ecological fallacy cannot be discarded from this analysis. The change in time sequence could not be stated in this cross-sectional study, but local doctors noted changes in the disease profile since the introduction of GM seeds and the massive use of glyphosate. The results of this study are also important because they describe a health problem in the environment where the people are living.

5. Conclusion

This research detected an urban environment severely polluted by glyphosate and other pesticides and identified high frequencies of cancer, suggesting a link between environmental exposure to glyphosate and cancer, although this was an

exploratory and observational design unable to make direct causal assertions. However, it is necessary to recognize the associations based on the analysis of the differences between exposure variables and high cancer prevalence, incidence, and mortality that must be verified with studies specifically designed for this purpose; further research is needed to reveal the exact relationship between cancer and glyphosate.

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Conflict of Interest

The authors declare they have no actual or potential competing financial interests.

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An Invitation of the Combination of the Geriatric Nutritional Risk Index and the Triglyceride to High-Density Lipoprotein Cholesterol Ratio as a Mortality Predictor in Maintenance Hemodialysis Patients

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Abstract

Background: The geriatric nutritional risk index (GNRI) has been developed as a tool to assess the nutritional risk. The triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio has been shown to be a predictor of cardiovascular (CV) outcomes in the general population. **Objectives:** The aim of this study was to determine whether the combination of GNRI and TG/HDL-C ratio is a predictor of all-cause mortality and CV deaths in maintenance hemodialysis (MHD) patients. **Methods:** We performed a retrospective, observational cohort study in which we enrolled 341 MHD patients from a single center in Japan who had been followed up for a mean of 48.0 ± 12.7 months. The outcomes were defined as the occurrence of all-cause mortality and CV deaths during the follow-up period. Baseline GNRI and TG/HDL-C ratios were investigated for associations with outcomes by using Cox proportion hazards models adjusted for demographic parameters. **Results:** Overall, 101 of the subjects had died, of whom 52 died due to CV events during the mean follow-up period of 48.0 ± 12.7 months. The patients were grouped into four categories according to a median GNRI < 95.3 or ≥95.3 and a median TG/HDL-C ratio < 2.09 or ≥2.09. The group with a GNRI ≥ 95.3 and TG/HDL-C ≥ 2.09 had significantly lower overall and CV mortality rate when compared with the other three groups. **Conclusion:** The combination of GNRI and TG/HDL-C ratio is an easily accessible marker for predicting all-cause mortality and CV deaths in MHD patients.

Keywords

GNRI, Triglyceride, HDL-Cholesterol, Mortality, Hemodialysis

1. Introduction

The geriatric nutritional risk index (GNRI), based on body weight, height and serum albumin level, has been identified as a simple method to evaluate nutritional condition of patients. Previous studies have demonstrated the reliability of GNRI in assessing malnutrition [1] and in predicting all-cause mortality in maintenance hemodialysis (MHD) patients [2] [3]. However, despite the significance of the GNRI on mortality in MHD patients in Western countries, the role of the GNRI on the nutritional condition has not been fully evaluated in Asian MHD patients.

Dyslipidemia, in which there is a combination of a high TG level and low HDL-C level, has been reported to strongly predict cardiovascular (CV) morbidity-coronary artery disease, in particular in the general population [4] [5]. Several studies have demonstrated a correlation between the TG to HDL-C (TG/HDL-C) ratios and both the severity of insulin resistance and presence of coronary atherosclerotic lesions in the general population [6] [7]. The TG/HDL-C ratio has been shown to predict the occurrence of myocardial infarction, ischemic heart disease [8] [9], and CV mortality, both in women with coronary artery disease and in the general population [10] [11].

The aim of this study was to determine if the combination of GNRI and TG/HDL-C ratio is a possible predictor of all-cause mortality and CV deaths in MHD patients.

2. Methods

2.1. Subjects and Protocol

This was a retrospective, observational cohort study conducted at a single center in Japan. The subjects were recruited from patients who had been routinely treated using an arteriovenous fistula in the dialysis unit of the Jyoban Hospital, Fukushima, Japan for at least 6 months. The Institutional Review Board of the Jyoban Hospital approved all study protocols, and they were performed in accordance with the Declaration of Helsinki guidelines regarding ethical principles for medical research involving human subjects. Informed consent was obtained from all of the subjects.

HD patients with malignancy, active inflammation, liver cirrhosis, gastrointestinal bleeding, cardiac valvular disease, or severe illness were excluded from participation and were transferred to another dialysis unit for intensive care. The patients who were enrolled as subjects (n = 341) underwent stable regular HD with a bicarbonate dialysate. Their underlying diseases of end-stage renal disease were diabetic nephropathy (n = 170), chronic glomerulonephritis (n = 154), hypertensive nephrosclerosis (n = 70), polycystic kidney disease (n = 8), and

chronic pyeonephritis (n = 3), or unknown origin (n = 6).

All patients were on thrice-weekly HD and no further selection was performed in patients. All of the subjects had an arteriovenous fistula. None of the subjects had residual renal function (urine volume ≥ 100 mL/day). Blood pressure (BP) was measured with a mercury sphygmomanometer with the patient in the supine position after resting for 10 to 15 minutes, and mean values for the 1-month period preceding enrollment were used in the statistical analysis. Dry weight was targeted to achieve a normotensive edema-free state. Previous cardiovascular disease and smoking status were collected from medical records. Diabetes was defined as a history or presence of diabetes and/or a fasting plasma glucose concentration > 126 mg/dl or HbA1c concentration > 6.5% or prescription of glucose-lowering agents.

Blood samples were taken prior to the first-week dialysis session day, following an overnight period without the consumption of food. Serum urea nitrogen, creatinine, calcium, phosphorous, albumin, total cholesterol, and C-reactive protein (CRP) levels and the hemoglobin concentration were measured with an autoanalyzer by standard laboratory methods. Total calcium was corrected for by the patient's albumin level. Intact parathyroid hormone (iPTH) was measured by an immunoradiometric assay. The body mass index (BMI) was expressed in kg/m^2. Weight was calculated as dry weight, defined as post-dialysis weight in which the patient was normotensive and with no signs of overhydration. Urea kinetics were assessed by measuring a blood-based dialysis parameter, Kt/V [12], and the mean value of the 3 measurements during each of the three months before the beginning of the study was used in the analysis. The normalised protein catabolism rate (nPCR) was used as an indirect indicator of protein intake and was obtained using the following formula as previously described [13].

The GNRI was calculated by modifying the Nutritional risk index for elderly patients, as reported by Yamada *et al.* [1] as follows:

$$\text{GNRI} = \left[14.89 \times \text{albumin } (\text{g/dl})\right] + \left[41.7 \times (\text{body weight/ideal body weight})\right].$$

For body weight, we considered the value at the end of the dialysis session, and it was also used for the calculation of BMI. Body weight/ideal body weight was set to 1 when the body weight of the patient exceeded the ideal body weight [14]. The ideal body weight in the present study was calculated using height and a BMI of 22, which is reportedly associated with the lowest morbidity rate in the Asian population [14].

Data for endpoints were obtained from hospital charts and through telephone interview with the patients, conducted by trained but double-blind interviewers. The primary endpoint of the study was all-cause mortality during the follow-up period between July 1, 2011 and July 31, 2016. The secondary endpoint was cardiovascular death, including those due to heart failure, myocardial infarction, arrhythmia, sudden death, and stroke. The vital status of the subjects was determined by searching the electronic dialysis records. Patients were censored if they were alive on July 31, 2016.

2.2. Statistical Analysis

Normally distributed, unpaired continuous values were expressed as means ± SD and compared by performing an Analysis of Variance (ANOVA). Nonparametric values were expressed as median values and compared by performing the Kruskal-Wallis test. Categorical values were expressed as percentages and compared by performing the Fisher's exact test.

The survival analysis was based on the Kaplan-Meier curve with subjects censored for death. A log-rank test was used to compare the survival rates of two groups. A multivariate Cox proportional hazards model with adjustment for multivariate factors was used to evaluate mortality risk. Results were expressed as a hazard ratio (HR) with 95% confidence intervals (CIs). A significant level of p value <0.05 was assumed to be statistically significant. All statistical analyses were performed by using the SAS version 9.2 software program (SAS Institute Inc., Cary, NC, USA) for Windows personal computers.

3. Results

Of the 341 included patients, we stratified the patients into four groups according to a median GNRI < 95.3 or ≥95.3 and a median TG/HDL-C ratio < 2.09 or ≥2.09. A comparison of the clinical characteristics of these groups is shown in Table 1. Compared to the patients with a GNRI < 95.3 (TG/HDL-C ≥ 2.09 or TG/HDL-C < 2.09), those with a GNRI > 95.3 (TG/HDL-C ≥ 2.09 or TG/HDL-C < 2.09) were older, had lower values of BMI, hemoglobin, serum phosphorus and albumin, and higher CRP values.

3.1. Risk of All-Cause Mortality

Overall, the mean follow-up period was 48.0 ± 12.7 months. During the follow-up period, 101 patients died (29.6%), including 52 due to CV diseases, 38 due to infectious diseases, 6 due to malignancy, and 5 due to gastrointestinal bleeding. Table 2 lists the HRs of variables for all-cause mortality. The univariate regression analysis shows that the group with a GNRI ≥ 95.3 and TG/HDL-C ≥ 2.09 had significantly lower overall mortality rate when compared with other three groups. In addition, older age, presence of diabetes, lower values of BMI, BP, hemoglobin, TSAT, phosphorus, intact-PTH and albumin, and higher CRP values were associated with a significant increase in overall mortality. In the multivariate analysis, the group with a GNRI ≥ 95.3 and TG/HDL-C ≥ 2.09, older age and lower TSAT were associated with overall mortality. Figure 1 illustrated the Kaplan-Meier survival curves for overall survival among the four study groups. The group with a GNRI ≥ 95.3 and TG/HDL-C ≥ 2.09 had better overall survival than those with a GNRI < 95.3 (TG/HDL-C ≥ 2.09 or TG/HDL-C < 2.09) (log-rank test, p < 0.0001).

3.2. Risk of CV Mortality

Of those who died due to CV causes during the follow-up period, 30 died due to heart failure, 11 due to cerebrovascular diseases, 6 due to myocardial infarction,

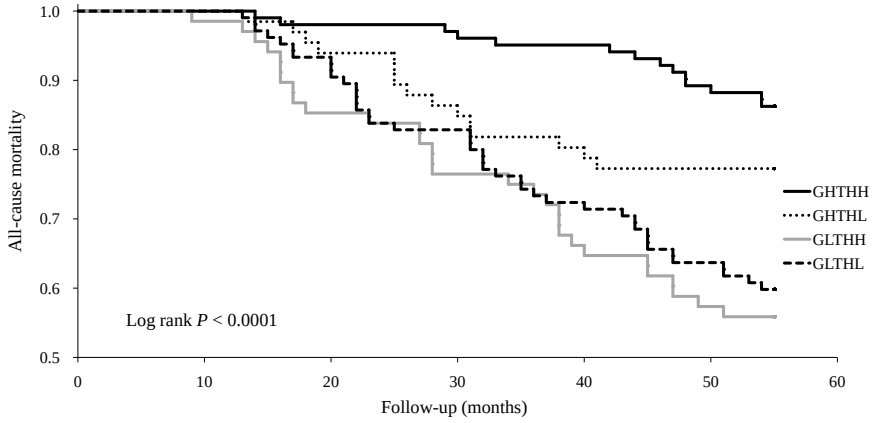

Figure 1. Kaplan-Meier analyses of overall survival among 4 study groups. GHTHH: GNRI ≥ 95.3 and TG/HDL ≥ 2.09; GHTHL: GNRI ≥ 95.3 and TG/HDL < 2.09; GLTHH: GNRI < 95.3 and TG/HDL ≥ 2.09; GLTHL: GNRI < 95.3 and TG/HDL < 2.09.

Table 1. Baseline characteristics of the study population.

	All patient (n = 341)	GNRI ≥ 95.3 and TG/HDL ≥ 2.09 (n = 102)	GNRI ≥ 95.3 and TG/HDL < 2.09 (n = 66)	GNRI < 95.3 and TG/HDL ≥ 2.09 (n = 68)	GNRI < 95.3 and TG/HDL < 2.09 N = 105	p-value
Age, years	65.5 ± 13.2	61.3 ± 11.8	62.4 ± 14.0	69.3 ± 13.0	69.0 ± 12.5	<0.0001
Female, %	36.4	30.4	31.8	41.18	41.9	0.2372
Dialysis vintage, years	5.1 ± 6.3	4.8 ± 6.4	5.0 ± 4.8	4.4 ± 6.0	5.6 ± 7.1	0.6002
Kt/V	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	0.3354
Diabetes, %	47.8	50.0	48.0	52.9	42.9	0.5798
History of previous CVD, %	10.6	11.8	12.1	7.4	10.5	0.7847
BMI, kg/m^2	22.2 ± 3.6	24.4 ± 3.2	23.3 ± 3.1	21.3 ± 3.7	20.1 ± 2.6	<0.0001
Systolic BP, mmHg	153.0 ± 17.2	153.6 ± 16.4	155.2 ± 17.5	152.7 ± 17.8	151.3 ± 17.6	0.5293
Diastolic BP, mmHg	80.1 ± 11.7	81.7 ± 10.6	81.1 ± 13.9	79.0 ± 11.9	78.5 ± 11.1	0.1748
Mean BP, mmHg	104.3 ± 12.3	105.6 ± 11.4	105.7 ± 13.8	103.6 ± 12.4	102.7 ± 11.9	0.2454
Pulse pressure, mmHg	73.0 ± 13.4	71.9 ± 12.2	74.2 ± 13.7	73.8 ± 14.3	72.9 ± 13.7	0.6924
Laboratory data						
nPCR, g/kg/day	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.2097
Hemoglobin, g/dl	10.7 ± 1.2	11.0 ± 1.1	10.8 ± 1.3	10.6 ± 1.1	10.4 ± 1.2	0.0083
Ferritin, ng/mL	87.0 ± 94.7	71.4 ± 85.9	85.4 ± 83.5	92.3 ± 86.4	99.7 ± 112.2	0.1798
TSAT, %	24.6 ± 12.6	23.5 ± 11.5	28.0 ± 13.6	23.3 ± 10.2	24.4 ± 13.9	0.0930
Calcium (corrected, mg/dl)	9.1 ± 0.6	9.1 ± 0.7	9.1 ± 0.6	9.2 ± 0.6	9.2 ± 0.6	0.5944
Phosphorus, mg/dl	4.9 ± 1.6	5.1 ± 1.5	5.3 ± 1.6	4.9 ± 1.7	4.6 ± 1.7	0.0130
Intact-PTH, pg/ml	128.4 ± 131.5	116.4 ± 96.3	142.9 ± 109.5	133.2 ± 177.3	127.9 ± 139.5	0.6306
Albumin, g/l	3.7 ± 0.3	3.9 ± 0.2	3.9 ± 0.2	3.5 ± 0.2	3.5 ± 0.3	<0.0001
CRP, mg/l	0.4 ± 0.8	0.3 ± 0.4	0.2 ± 0.4	0.5 ± 0.9	0.5 ± 1.0	0.0311
Total cholesterol, mg/dl	151.2 ± 30.5	156.5 ± 30.9	142.2 ± 28.9	153.4 ± 26.2	150.1 ± 32.6	0.0245
Triglyceride (TG), mg/dl	114.4 ± 74.2	172.2 ± 78.3	65.6 ± 23.6	145.6 ± 75.6	68.9 ± 21.9	<0.0001
HDL-C, md/dl	46.2 ± 14.2	36.3 ± 7.4	54.1 ± 13.2	38.7 ± 10.2	55.7 ± 12.9	<0.0001
Non-HDL-C, mg/l	105.0 ± 30.2	120.2 ± 29.0	88.1 ± 25.5	114.7 ± 22.9	94.4 ± 28.9	<0.0001

Continuous variables are expressed as means ± SD. Count data are expressed as percentages. Abbreviations: *CVD* cardiovascular disease, *GNRI* geriatric nutritional risk index, *BMI* body mass index, *BP* blood pressure, *nPCR* normalized. Protein catabolic rate, *TSAT* transferrin saturation, *PTH* parathyroid hormone, *CRP* C-reactive protein, *HDL-C* high-density lipoprotein cholesterol.

Table 2. Relation of study groups to all-cause mortality using Cox proportional hazards model.

All-cause mortality	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
	Study Group			
GNRI ≥ 95.3 and TG/HDL ≥ 2.09	1.00	-	1.00	-
GNRI ≥ 95.3 and TG/HDL < 2.09	1.84 (0.88 - 3.86)	0.1020	2.24 (1.06 - 4.76)	0.0340
GNRI < 95.3 and TG/HDL ≥ 2.09	4.06 (2.19 - 7.90)	<0.0001	2.56 (1.24 - 5.48)	0.0105
GNRI < 95.3 and TG/HDL < 2.09	3.53 (1.98 - 6.71)	<0.0001	2.40 (1.19 - 5.00)	0.0136
Age	1.06 (1.04 - 1.08)	<0.0001	1.05 (1.03 - 1.07)	<0.0001
Female sex	0.93 (0.61 - 1.39)	0.7371		
Dialysis vintage	0.99 (0.95 - 1.02)	0.4544		
Kt/v	0.49 (0.22 - 1.10)	0.0829		
Diabetes	1.61 (1.09 - 2.41)	0.0166	1.54 (1.02 - 2.34)	0.0382
History of previous CVD, %	1.02 (0.51 - 1.82)	0.9588		
BMI, kg/m^2	0.89 (0.84 - 0.95)	0.0001		
Systolic BP, mmHg	0.99 (0.98 - 1.00)	0.0120		
Diastolic BP, mmHg	0.97 (0.95 - 0.98)	<0.0001	1.00 (0.98 - 1.02)	0.8761
Mean BP, mmHg	0.97 (0.96 - 0.99)	0.0002		
Pulse pressure, mmHg	1.00 (0.99 - 1.02)	0.8301		
	Laboratory data			
nPCR, g/kg/day	0.35 (0.12 - 1.04)	0.0586		
Hemoglobin, g/dl	0.85 (0.73 - 0.99)	0.0401	1.02 (0.85 - 1.22)	0.8461
Ferritin, ng/ml	1.00 (1.00 - 1.00)	0.7640		
TSAT, %	0.97 (0.96 - 0.99)	0.0029	0.97 (0.95 - 0.99)	0.0067
Calcium, mg/dl; corrected	1.21 (0.90 - 1.61)	0.2090		
Phosphorus, mg/dl	0.88 (0.78 - 1.00)	0.0466	0.99 (0.87 - 1.14)	0.9294
Intact-PTH, pg/ml	1.00 (1.00 - 1.00)	0.0451	1.00 (1.00 - 1.00)	0.3671
Albumin, g/l	0.27 (0.16 - 0.47)	<0.0001	0.81 (0.35 - 1.95)	0.6367
CRP, mg/l	1.26 (1.03 - 1.48)	0.0267	1.03 (0.83 - 1.24)	0.7391
Total cholesterol, mg/dl	1.00 (0.99 - 1.00)	0.6643		
Triglyceride, mg/dl	1.00 (0.99 - 1.00)	0.0064		
HDL-C, md/dl	1.01 (1.00 - 1.02)	0.1837		
Non-HDL-C, mg/l	1.00 (0.99 - 1.00)	0.2860		

and 5 due to arrhythmia. The Cox proportional hazard regression analysis of the four groups for CV mortality is shown in Table 3. The univariate regression analysis shows that the group with a GNRI ≥ 95.3 and TG/HDL-C ≥ 2.09 had significantly lower CV mortality rate when compared with other three groups. In addition, older age, lower mean BP and lower albumin were associated with CV mortality. In the multivariate analysis, the group with a GNRI ≥ 95.3 and TG/HDL-C ≥ 2.09, older age and lower mean BP were associated with CV mortality. Figure 2 shows the Kaplan-Meier survival curves for CV mortality among the four study groups. The group with a GNRI ≥ 95.3 and TG/HDL-C ≥ 2.09 had better CV survival than those with other three groups.

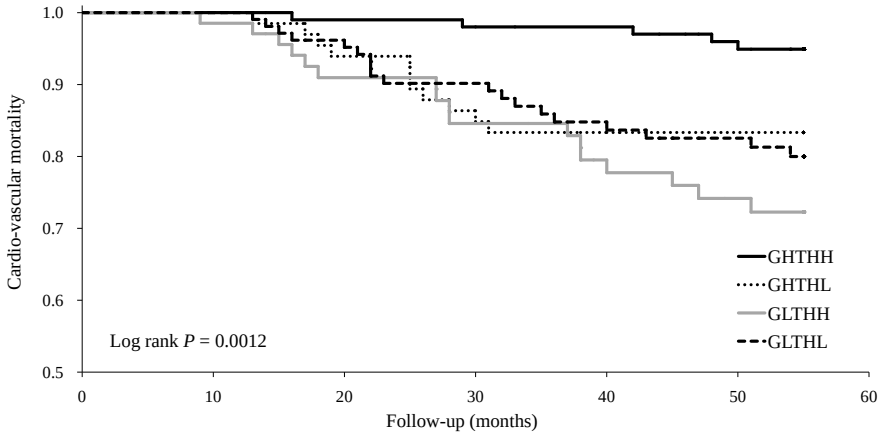

Figure 2. Kaplan-Meier analyses of cardiovascular survival among 4 study groups. GHTHH: GNRI ≥ 95.3 and TG/HDL ≥ 2.09; GHTHL: GNRI ≥ 95.3 and TG/HDL < 2.09; GLTHH: GNRI < 95.3 and TG/HDL ≥ 2.09; GLTHL: GNRI < 95.3 and TG/HDL < 2.09.

Table 3. Relation of study groups to cardiovascular mortality using Cox proportional hazards model

Cardiovascular death	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
	Study Group			
GNRI ≥ 95.3 and TG/HDL ≥ 2.09	1.00	-	1.00	-
GNRI ≥ 95.3 and TG/HDL < 2.09	3.72 (1.35 - 11.80)	0.0105	3.83 (1.39 - 12.17)	0.0092
GNRI < 95.3 and TG/HDL ≥ 2.09	6.25 (2.47 - 19.01)	<0.0001	3.56 (1.24 - 11.89)	0.0178
GNRI < 95.3 and TG/HDL < 2.09	4.35 (1.75 - 13.12)	0.0011	2.55 (0.90 - 8.40)	0.0796
Age	1.06 (1.04 - 1.09)	<0.0001	1.04 (1.01 - 1.07)	0.0043
Female sex	1.10 (0.62 - 1.91)	0.7339		
Dialysis vintage	0.97 (0.91 - 1.02)	0.1979		
Kt/v	0.34 (0.12 - 1.04)	0.0579		
DM	1.61 (0.94 - 2.83)	0.0857		
History of previous CVD, %	0.69 (0.21 - 1.68)	0.4445		
BMI, kg/m^2	0.94 (0.87 - 1.02)	0.1590		
Systolic BP, mmHg	0.97 (0.96 - 0.99)	0.0010		
Diastolic BP, mmHg	0.96 (0.93 - 0.98)	0.0001		
Mean BP, mmHg	0.96 (0.94 - 0.98)	<0.0001	0.97 (0.95 - 1.00)	0.0210
Pulse pressure, mmHg	0.99 (0.97 - 1.01)	0.2846		
	Laboratory data			
nPCR, g/kg/day	0.23 (0.05 - 1.07)	0.0609		
Hemoglobin, g/dl	0.89 (0.72 - 1.12)	0.3121		
Ferritin, ng/ml	1.00 (1.00 - 1.00)	0.7979		
TSAT, %	0.99 (0.96 - 1.01)	0.2137		
Calcium, mg/dl; corrected	1.31 (0.86 - 1.93)	0.2007		
Phosphorus, mg/dl	0.88 (0.74 - 1.04)	0.1323		
Intact-PTH, pg/ml	1.00 (0.99 - 1.00)	0.0704		
Albumin, g/l	0.30 (0.14 - 0.67)	0.0036	0.61 (0.19 - 2.08)	0.4277
CRP, mg/l	1.30 (1.00 - 1.60)	0.0536		
Total cholesterol, mg/dl	1.00 (0.99 - 1.01)	0.8668		
Triglyceride, mg/dl	1.00 (0.99 - 1.00)	0.3844		
HDL-C, md/dl	1.01 (0.99 - 1.03)	0.2887		
Non-HDL-C, mg/l	1.00 (0.99 - 1.01)	0.5009		

4. Discussion

The results of this study showed that the combination of the GNRI and the TG/HDL-C ratio was predictive of all-cause mortality and CV deaths in MHD patients. Stratification of the severity of malnutrition and dyslipidemia in MHD patients should be recognized as a reliable available tool for predicting long-term survival of MHD patients.

The assessment and monitoring of protein and energy nutritional statuses are essential in the prevention, diagnosis and treatment of uremic malnutrition in dialysis patients [15]. As no definitive single, gold-standard test is currently available to assess nutritional status, a number of different tools are required. Many nutritional screening tools have been developed for the elderly, children, hospitalized patients, community patients, and/or patients with cancer or infections. Some of them may be safely and easily applied to patients on maintenance HD as well [16]. The Subjective Global Nutritional Assessment is a well validated clinical tool for screening malnutrition [17] and the malnutrition-inflammation score (MIS) is able to predict mortality and hospitalization in maintenance HD patients [18]. However, both require subjective assessment and judgment by a skilled examiner.

The GNRI consists of few objective components, including serum albumin and BMI and represents a simple nutritional screening tool. Takahashi *et al.* [19] have recently demonstrated that GNRI at initiation of HD therapy could predict CV mortality with incremental predictability compared to serum albumin and BMI in HD patients. Although the lowest GNRI quartile (<92) is strongly associated with malnutrition signs and in addition to an increased risk of overall mortality, no predictive value emerged regarding non-fatal CV events in HD patients. In previously conducted study, we have demonstrated that GNRI was one of the parameters as a predictor of overall mortality [20]. However, CV mortality was not associated with GNRI values, and did not differ among the GNRL quartiles. The GNRI can be considered a simple and reliable marker of predictor for mortality risk in Japanese MHD patients. The GNRI has been shown able to predict increased future healthcare costs and a higher risk of hospitalization in independent-living older adults; so it can be considered to be a rapid and low-cost tool that has the potential to be routinely used in regular population-based settings.

HD patients have unique lipid profiles, and the associations between their lipid profiles and CV outcomes and mortality are different from the associations in the general population [21]. Kilpatrick *et al.* [21] demonstrated that HDL-C and TG did not to predict CV or all-cause mortality in a large HD cohort who were followed up for 3 years. We previously used the TG/HDL-C ratio to predict all-cause mortality and CV events, and the results clearly demonstrated the predictive power of the TG/HDL-C ratio in MHD patients [22]. Chen *et al.* [23] have recently demonstrated the predictive ability of the TG/HDL-C ratio for CV outcomes and survival in patients undergoing prevalent dialysis. The TG/HDL-C ratio may therefore be the optimal marker for predicting CV outcomes in

MHD patients.

The mechanisms underlying the association between the combination of GNRI and TG/HDL-C ratios and all-cause mortality and CV deaths in MHD patients are still unknown. Multiple mechanisms may explain the link between malnutrition and mortality in renal failure, including derangements in muscle, adipose tissue, gastrointestinal, hematopoietic and immune systems, and abnormal activation of the inflammatory process [24], in addition to co-morbidities. Low albumin, cholesterol, and BMI are indicators of protein-energy wasting (PEW), but they may not be causally responsible for the negative outcome. The reduction in muscle mass, namely sarcopenia, observed in PEW may be due to uremic toxins or procatabolic conditions (metabolic, hormonal, or neuropathic derangements, including inactivity). In turn, muscle wasting may lead to reduced skeletal, respiratory, and cardiac muscle function, causing functional insufficiency and then risk of severe events [24].

We have previously reported an interaction between the TG/HDL-C ratio and DM in the prediction of CV events [22]. This finding is also consistent with the interaction exists between them in predicting events in the general population, and patients with a high TG/HDL-C ratio have been reported to be predisposed to diabetes mellitus [25]. The diabetic status of patients with both diabetes and dyslipidemia is crucial when assessing CV outcomes compared with non-diabetic patients with dyslipidemia. As shown in a clinical study [26], the manipulation of high TG and low HDL-C levels in diabetic patients by medical interventions does not reduce their risk of CV outcomes. Consequently, the utility of the TG/HDL-C ratio for predicting long-term CV outcomes in diabetic MHD patients needs to be carefully assessed in further large-scale investigations.

The present study had several limitations. Firstly, the observational nature of the study precludes drawing conclusions about causal relationships. Secondly, the measurement of alternative nutritional markers and the specific lipoproteins related to atherogenic dyslipidemia was not taken, and as such, the relationship between TG/HDL-C ratios and apolipoproteins in MHD patients needs to be investigated further. Thirdly, this was a single-center study, and all of the participants were Japanese in addition to being treated by the same physicians. The same uniform laboratory tetsts were performed during the observation period, which guaranteed the accuracy of our results, but our conclusions cannot be generalized to other ethnicities.

5. Conclusion

The results of this study suggest that the combination of the GNRI and the TG/HDL-C ratio can independently predict all-cause mortality and CV deaths in MHD patients, especially in diabetic MHD patients.

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Disclosure

All authors have no conflicts of interest to declare.

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To Determine the Effects of Labor Induction on Maternal and Fetal Outcome in Postterm Pregnancies (41 Weeks Plus)

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Abstract

Background: Pregnancies progressing postterm are associated with a higher perinatal morbidity and mortality rates than those delivered at term. In a United Kingdom study, the rate of stillbirth increased from 0.35 in 1000 live births in pregnancies of 37 weeks to 2.12 in 1000 live births in pregnancies of 43 weeks gestation. Morbidities associated with postterm births include an increased risk of fetal distress, intrauterine growth restriction, dysfunctional labor, shoulder dystocia, obstetric trauma (relative risk 1.09 - 1.68) and an increase in perinatal complications, such as aspiration of meconium and asphyxia, peripheral nerve injury, greenstick bone fractures, pneumonia and septicemia (adjusted odds ratio 1.4 - 2.0). Antenatal surveillance and induction of labor may decrease the risks of an adverse outcome. In a recent review of term and postterm pregnancies in Norway, we found that there were adverse outcomes associated with both postterm pregnancy and induction of labor independently. On comparison of the two, a randomized controlled trial showed no difference in their neonatal outcome, but demonstrated a reduction in the cesarean delivery rate when labor was induced at 41 weeks. **Aim of the Work:** The aim of this study was to determine the effect of labor induction on maternal and fetal outcome in postterm pregnancies. **Subjects and Methods:** This study was carried out on 150 pregnant women who had completed 41 weeks of gestation between Jun. 1, 2012 up to Dec. 31, 2012 at *Department of Obstetrics & Gynecology*, *Faculty of Medicine*, *Benghazi University*, and were scheduled for induction of labor after cardiotocography (CTG) and ultrasonography (USS) have been done and Bishop's score assessed, to determine the effects of labor induction on maternal and fetal outcome in postterm pregnancies (41 weeks plus). **Results:** Regarding the relationship between a history of (H/O) postdatism and fetal distress, it was found that there was no significant relationship between them. There was a significant relationship between a history of macrosomia and fetal distress. There was a

significant relationship between instrumental delivery and fetal distress. The majority of the fetal distress had an indication for Caesarean section (CS) (fetal distress (FD) and fetal distress meconium (FDM) more than those without fetal distress. All fetuses that had APGAR scores of 8 were distressed. There was a significant relationship between the APGAR score at 10 minutes with fetal distress. All fetuses that had meconium aspiration had fetal distress. There was a significant increase in the amount of oxytocin in unit in distressed cases than the non-distressed ones. The total duration of induction was also significantly increased in stressed fetuses than the non-stressed ones. There was a significant increase in the weight of distressed fetuses than the non-distressed. **Conclusions:** In conclusion, there was no difference in the neonatal outcome or mode of delivery for postterm pregnancies managed either by immediate induction of labor or expectantly with serial antenatal surveillance. The outcomes were generally good, and neonatal morbidity, cesarean section, and operative vaginal delivery rates were low. If pregnancy is uncomplicated and continued surveillance is possible, women's own wishes may guide the decision to induce or monitor a pregnancy beyond 41 weeks.

Keywords

Postterm Pregnancy, Mortality Rate

1. Introduction

There is an increased risk of fetal and neonatal morbidity and mortality in postterm pregnancies [1] as well as an increased maternal morbidity [2]. Stillbirths occurring at and beyond term (37 - 42 weeks gestation) are a major public health problem contributing more to perinatal mortality than either deaths from complications of prematurity or the sudden infant death syndrome [3]. Induction of labour at term is used to prevent this high fetal mortality from postterm pregnancies; however, both clinicians and patients alike are concerned about the its risks, which include uterine hyper-stimulation, failed induction and increased Caesarean section rates. Postterm pregnancies are also associated with increased costs which arise from antenatal monitoring of the foetus and the cost of inducing labour [4], in addition to being a source of significant anxiety for the pregnant woman [5]. Optimization of these conflicting pressures still poses a clinical challenge.

A normal term pregnancy is between 37 and 42 weeks of gestation with a progressive increase in the perinatal morbidity and mortality rates during this period. In clinical practice, it is important, yet very difficult to define an "ideal" time when the benefits of a medical intervention (induction of labor) outweigh the benefits of the natural evolution of pregnancy. Both preterm (defined as delivery before 37 completed weeks of gestation) and postterm (delivery at or beyond 41 week of gestation) births are associated with increased neonatal morbidity and mortality. Postterm pregnancy constantly remains a difficult and

controversial problem in modern obstetrics. Postterm pregnancy, by definition, refers to a pregnancy that has extended to or beyond 41 weeks of gestation (294 days, or estimated date of delivery (EDD) + 14 days), and it complicates 10% of all gestations [6] [7]. Accurate dating of the pregnancy is critical to the diagnosis [6]. The most frequent cause of an apparently postterm pregnancy is an error in dating. When postterm pregnancy truly exists, the cause is usually unknown [6]. Postterm antenatal fetal surveillance traditionally begins at 42 completed weeks of gestation; however, recent data have shown that a significant percentage of cases of perinatal asphyxia occur between 40 and 42 weeks of gestation. Some studies [8] [9] mentioned that the pregnancy risks start increasing from gestational week 41. In this paper, we have reviewed the literature data about the diagnosis of postterm pregnancy, prenatal indicators, and maternal fetal outcomes of prolonged pregnancy, and the timing to start the antepartum fetal tests.

The aim of the study was to determine the effects of labor induction on maternal and fetal outcome in postterm pregnancies (41 weeks plus) of patients attending the obstetrics department of Aljamhoria Hospital, Benghazi and to evaluate the outcome of mother and fetus.

2. Subjects and Methods

2.1. Subjects

A total of 150 women who reached 41 weeks + gestation between June 1, 2012 and Dec 31, 2012 at the *Department of Obstetrics & Gynecology*, *Faculty of Medicine*, *Benghazi university* and who were scheduled for induction of labor after a normal CTG and USS and a favourable Bishop's score were included in this prospective observational study.

Caesarean delivery rates were calculated for those who underwent induction.

2.2. Exclusion Criteria

Women with any medical or obstetric risk factors were excluded, e.g. previous CS, women aged more than 35 years, multipara (>4 delivery), multiple pregnancy, any medical disease (hypertension or diabetes) and unreactive CTG or biophysical profile (BPP).

3. Methods

Women assigned to both arms of the study had the same baseline assessment: an ultrasound scan (with emphasis on estimated fetal weight and amniotic fluid volume), a cardiotocogram, and a clinical vaginal examination. For women assigned to continued antenatal assessment, induction of labor was arranged if the cardiotocogram recordings were abnormal, the estimated fetal weight was less than 2 standard deviations, or oligohydramnios (amniotic fluid index less than 5 cm or single deepest pocket less than 2 cm) was found. If these investigations are normal, they were reassessed every third day until spontaneous delivery occurred, or until labor was induced on day 300.

Women who had a favorable cervix (Bishop score of 6 or more) were induced by amniotomy followed by oxytocin Syntocinon, infusion. Women with an unfavorable cervix (Bishop score less than 6) had cervical priming using misoprostol (prostaglandin E_1 50 mcg pessary encased in a gelatin capsule) at 6-hour intervals in the posterior fornix. A maximum of four doses were given in a 24-hour period, and cervical priming was continued for a maximum of 2 days. Once the cervix was favorable, amniotomy and oxytocin infusion were used. Women with a uterine scar were induced with 0.5 mg dinoprostone (prostaglandin E_2, Minprostin endocervical gel) given intracervically every 12 hours.

Women being induced, or being assessed on admission in labor, had a cardiotocogram. If this was abnormal, or if meconium was seen after rupture of membranes, then a continuous electronic fetal monitoring was recommended. Antenatal, intrapartum, and postnatal data were collected on a single chart that accompanied the patient through this period and was completed by the staff contemporaneously. The charts were designed to allow automatic optical recognition and transmission to a computer database based in the University Clinical Trials Office. The process of data transmission was checked for error by two of the authors (R.H. and E.S.).

3.1. The Outcome of the Neonate Was Recorded

Meconium aspiration, Birth asphyxia, neonatal (NN) death, Still birth.

Neonatal morbidity was primarily defined by assessing a series of relevant outcomes. Information about the presence of meconium, birth weight, crown-heel length, Apgar scores, umbilical cord pH, and base excess and the need for resuscitation were recorded immediately after delivery. Supplementary medical information was obtained from the routine pediatric examination on the first or second day, and if admitted, from the neonatal intensive care unit (NICU).

Neonatal morbidity was scored by evaluating the degree of deviation from the potential of a perfect outcome for each newborn. We defined a perfect outcome as being an infant with a birth weight of 3.8 kg and a Ponderal Index of 2.88 (a measure of weight relative to length obtained from 180 healthy newborns with gestational age 287 days or more acting as controls in a study of preeclampsia). Other optimal features for outcome were considered to be 1- and 5-minute Apgar scores of 10, umbilical cord pH of 7.40 with base excess equal to 0 (zero), and no medical complications or need for treatment. To compare the study groups, we assigned a priori weights to the outcome variables based on clinical judgment and consensus among the researchers. The sum in each neonate constitutes a Neonatal Morbidity score, which increases with increasing morbidity. One Neonatal Morbidity unit corresponds to, for example, the presence of meconium, a one-point decrease in the 5-minute Apgar score, or a pH decrease of 0.1. We further calculated the Canadian Multicenter postterm Pregnancy neonatal morbidity index developed by Hannah *et al.* [9], in which morbidity was defined when the upper 2 percentile was exceeded.

Severe perineal lacerations were defined as third and fourth-degree perineal

lacerations during delivery. Maternal hemorrhage was defined as blood loss more than 500 ml at delivery. Uterine contraction abnormalities were defined as prolonged first stage of labor (less than 1 cm cervical dilatation per 1.5 hours), prolonged active second stage of labor (longer than 1 hour) and short active second stage of labor (less than 15 minutes). Precipitate labor was defined as a total length of labor less than 3 hours.

3.2. Ethical Considerations

- Approval was taken from the ethics committee.
- Each participant was informed about the aim and procedures of the study and consent to participate in the study was obtained.

4. Results

This study was carried out on 150 pregnant women who reached more than 41 weeks' gestation between Jun 1, 2012 and Dec 1, 2012 and who were scheduled for induction of labor after a normal CTG and USS and a favourable Bishop's score, to determine the effects of labor induction on maternal and fetal outcome in postterm pregnancies (41 weeks plus).

Table 1 shows the incidence of caesarean section and fetal distress among the studied group, it was found that a caesarean section was done in 17 cases (11.3%) and fetal distress was found in 16 cases (10.7%).

Table 2 shows the relationship between nationality and fetal distress. It was found that there was no significant association between nationality and fetal distress ($p > 0.05$).

Table 1. Distribution of the studied patients regarding the caesarean delivery and fetal distress.

	Number	Percent
Caesarean section	17	11.3
Fetal distress	16	10.7
Normal delivery	117	
Total study group	150	100.0

Table 2. Nationality in relation to fetal distress.

			Fetal distress		Total
			No N = 134	Yes N = 16	
Nationality	**Libyan**	No	127	15	142
		%	94.8%	93.8%	94.7%
	Non Libyan	No	7	1	8
		%	5.2%	6.3%	5.3%
X^2			0.030.		
p			604		

Table 3 shows the relationship between blood group and fetal distress. It was found that there was a significant relationship between blood group B and O RH- and the incidence of fetal distress ($p < 0.05$).

Table 4 shows the relationship between booking status and fetal distress. It was found that there was no significant relationship between booking status and fetal distress.

Table 5 shows the relationship between H/O postdatism and some other variables. It demonstrates that there was no significant relationship between H/O of postdatism and fetal distress ($p > 0.05$). All the patients that had H/O macrosomia had fetal distress, showing a significant relationship between the two ($p < 0.05$). The majority of fetal distress cases were from caesarean section delivery, indicating a significant relationship between CS and fetal distress ($p < 0.01$).

Table 3. Blood group and RH group in relation to fetal distress.

BL GR RH+		Fetal distress		Total	X^2
		No	Yes		*p*
A	No	32	3	35	1.103 0.894
	%	23.9%	18.8%	23.3%	
AB	No	12	2	14	
	%	9.0%	12.5%	9.3%	
B	No	19	3	22	
	%	14.2%	18.8%	14.7%	
O	No	45	4	49	
	%	33.6%	25.0%	32.7%	
BL GR RH-					
A	No	11	0	11	18.670 0.001*
	%	8.2%	.0%	7.3%	
AB	No	3	0	3	
	%	2.2%	.0%	2.0%	
B	No	0	2	2	
	%	.0%	12.5%	1.3%	
O	No	12	2	14	
	%	9.0%	12.5%	9.3%	

Table 4. Booking of the pregnancy in relation to fetal distress.

			Fetal distress		Total
			No N = 133	Yes N = 16	
Booked	No	No	1	0	1
		%	0.7%	0.0%	0.7%
	Yes	No	133	16	149
		%	99.3%	100.0%	99.3%
X^2			0.120		
p			0.893		

Table 5. Relationship between relevant history and other variables.

History of (H/O) postdatism			Fetal distress		Total	X^2 *P*
			No	Yes		
	No	No	116	15	131	667 0.366
		%	86.6%	93.8%	87.3%	
	Yes	No	18	1	19	
		%	13.4%	6.3%	12.7%	
H/O Macrosomia	No	No	134	14	148	16.76 0.011*
		%	100.0%	87.5%	98.7%	
	Yes	No	0	2	2	
		%	.0%	12.5%	1.3%	
MOD CS	CS	No	6	11	17	58.758 0.001*
		%	4.5%	68.8%	11.3%	
	Normal	No	128	5	133	
		%	95.5%	31.3%	88.7%	
CS	Emergency	No	4	9	13	6.672 0.048*
		%	30.7%	69.3%	6.7%	
	Elective	No	2	2	4	
		%	50.0%	50.0%	2.7%	
Indication CS	Failed induction	No	1	0	1	38.34 0.0001*
		%	0.7%	0.0%	0.7%	
	Fetal distress	No	5	4	9	
		%	3.7%	25.0%	6.0%	
	Fetal distress & meconium	No	0	2	2	
		%	0.0%	12.5%	1.3%	
Apgar score 10 min	8.00	No	0	5	5	45.958 0.0001*
		%	0.0%	31.3%	3.3%	
	9.00	No	70	9	79	
		%	52.2%	56.3%	52.7%	
	10.00	No	64	2	66	
		%	47.8%	12.5%	44.0%	
SEX	Female	No	97	8	105	3.412 0.063
		%	72.4%	50.0%	70.0%	
	Male	No	37	8	45	
		%	27.6%	50.0%	30.0%	

The relationship between type of CS and fetal distress found that the emergency CS showed a high percentage of fetal distress, ($p < 0.05$).

The relationship between the indication for CS and fetal distress found that the majority of the fetal distress cases had an indication for CS (FD, and FDM)

more than the non-fetal distress indication ($p < 0.01$).

It was found that all fetuses that had Apgar scores of 8 were distressed, showing a significant relationship between Apgar score at 10 min and fetal distress ($p < 0.001$).

There was no significant relationship between fetal distress and sex of the fetus.

There was a significant association between the respiratory distress syndrome and fetal distress ($p < 0.01$).

Table 6 shows the relationship between fetal distress and different risk factors. Regarding duration of hospital stay, it was found that there was a significant increase in hospital stay period in fetal distress cases than the others. Gestational age was found not to have any significant effect on the inncidence of fetal distress. The number of props given did not show any association to fetal distress neither did the duration from start of induction to transfer to labour room.

There was a significant increase in the amount of oxytocin in unit in distressed cases than the non-distressed cases ($p < 0.05$), and the total duration of induction, also showed a significant increase in stressed fetuses than the non-stressed ($p < 0.05$).

The level of haemoglobin (Hb) before and after delivery showed no significant relationship to fetal distress ($p > 0.05$). There was a significant increase in the weight of distressed fetuses than the non-distressed ones ($p < 0.01$).

5. Discussion

In our study, it was found that there was a significant relationship between blood group B and O RH- and the incidence of fetal distress.

In agreement with our study, Denomme *et al*., (2004) showed that there was a significant positive correlation between maternal ABO-mismatched blood and fetal distress [10].

Table 6. Relationship between fetal distress and different risk factors.

	Without fetal distress	With fetal distress	t-test	*p*
DAY OF STAY IN HOSPITAL	2.6716 ± 1.11	3.75 ± 1.183	13.186	0.0001*
GA (WEEKS)	42.21 ± 0.22	42.31 ± 0.30	0.21	0.62
No of Props given	2.1866 ± 0.75	2.500 ± 0.73	2.464	0.119
Duration from start of induction to transfer to Labor room	2.2162 ± 0.75	2.4667 ± 0.63	1.499	0.223
Amount of oxytocin in unit	9.5224 ± 5.58	13.2500 ± 5.56	6.362	0.013*
Total Duration of induction and labor	2.7090 ± 1.05	3.6875 ± 0.83	3.064	0.042*
Hb level before delivery	11.10 ± 0.50	10.50 ± 0.44	1.017	0.085
Hb level after delivery	9.9366 ± 0.50	9.9250 ± 0.51	0.008	0.931
WT/gm	3288.13 ± 334	3870.31 ± 271.29	44.778	0.0001*

In our study, it was found that there was no significant relationship between booking status and fetal distress. In disagreement to our study, however, Jaspinder and Kawaljit (2012) demonstrated that a higher incidence of fetal distress was seen in unbooked mothers (61.12%) when compared to booked mothers (38.89%) [11].

A recent study conducted by Khatoon *et al.* showed that one of the most common reasons for referral among unbooked women was fetal distress, evidenced by meconium stained liquor. The high incidence rate of birth asphyxia due to Fetal Distress prior to birth in unbooked mothers has been reported by various authors in their research works. It reveals that young age and an unbooked status might had withdrawn them from taking antenatal care at an early gestational age or till the development of obstetric complications which had led to the development of antepartum or intrapartum fetal hypoxia and distress [12].

In this study, it was found that there was a significant relationship between H/O macrosomia and CS with fetal distress. Jaspinder and Kawaljit (2012) found that emergency Caesarean section occurred in 79.17% of mothers with Fetal Distress [11].

In our study, we found that all fetuses that had an Apgar score of 8 were distressed indicating a significant relationship between Apgar score at 10 min and fetal distress. Mojbian *et al.* (2013) found that there were statistically significant differences between APGAR score and fetal distress [13].

In our study, there was no significant relationship between fetal distress and sex of the fetus.

In this study, all the fetuses that had meconium aspiration had fetal distress, showing a significant association between the respiratory distress syndrome and fetal distress. There was a significant increase in hospital stay period in fetal distress cases than the others. It was found that there was no significant difference between the two studied groups regarding gestational age.

There was a significant increase in the amount of oxytocin in unit in distressed cases than the non-distressed. The total duration of induction also shows a significant increase in stressed fetuses than the non-stressed.

There was no significant relationship between Hb level and fetal distress. Jaspinder and Kawaljit (2012) showed that anemia was associated with the highest incidence of Fetal Distress (34.73%). Oligohydraminos, Pregnancy Induced Hypertension and Intrauterine Growth Retardation were responsible for Fetal Distress in 19.45%, 18.06% and 18.06% of cases, respectively. The various other obstetric conditions implicated in a decreasing order were: Meconium Stained Amniotic Fluid (16.67%), Preterm Labor with Scar tenderness (16.67%), Preterm Premature Rupture of Membrane (12.50%), Postdatism (12.50%), Placenta Previa (09.73%), Uteroplacental Insufficiency (06.95%), True Nuchal Knot (06.95%), Failed Labour (05.56%) and Gestational Diabetes mellitus (02.78%) [11].

In this study, the weight of the baby was directly proportional to the incidence of fetal distress.

This study shows that policies of immediate induction of labor or serial antenatal monitoring while awaiting spontaneous labor produce no significant differences in the neonatal outcome or mode of delivery of postterm pregnancies. Women who were induced were more likely to have a precipitate labor with a shorter active second stage, although these factors did not alter neonatal outcome.

The study of postterm pregnancies is complicated by the fact that both the normal duration of pregnancy and the best method to define the estimated delivery of pregnancy remain controversial. In Norway, the normal duration of pregnancy is defined as 282 days, in line with several studies examining the mean and mode of pregnancy duration. Similarly, ultrasonography has been shown to be the method of choice for defining the estimated date of delivery (where equipment and trained personnel are available) and is found to reduce the number of pregnancies defined as being postterm [14] [15]. In Norway, pregnancies are routinely dated by ultrasonography at 18 weeks and we defined the point for investigating the postmature pregnancy as being one complete week beyond the normal duration of pregnancy, *i.e.*, 289 days.

Several studies have demonstrated that the risks of stillbirth and perinatal mortality increase beyond 41 weeks of gestation [16] [17]. Despite this, the absolute mortality rate remains quite low, and it would take at least 500 inductions at 41 weeks to prevent one neonatal death [5]. Consequently, the maternal and fetal morbidity related to induction of labor are potentially important issues and were the subject of investigation here. This study was not designed to examine differences in mortality. Indeed, there were no fetal deaths in the study, and the only neonatal death related to birth asphyxia was secondary to a true knot in the cord. This death would probably not have been avoided by induction a few days earlier [17].

In the process of designing this study we reviewed the methodology used by other investigators to define neonatal morbidity. We found most scoring systems unsuitable for a quantitative comparison of the study groups [18], among other things because they focus on premature infants, NICU admission, and mortality. The Neonatal Morbidity score was based on suboptimal outcomes which have been described as being associated with postterm delivery. This scoring system was tested in two pilot studies before the randomized trial. To quantify neonatal outcome, we constructed the "perfect infant" and defined multiple criteria to assess the deviation from this. The weight ascribed to various criteria could be debated, but the fundamental design of the trial—including randomization and a priori definitions—ensures a valid group comparison. The morbidity index used by Hannah *et al.* [19] was also calculated, but the index is difficult to interpret clinically and provided no further information on neonatal morbidity.

Mothers who had Preterm Premature Rupture of Membrane (PPROM) further experienced Fetal heart rate abnormalities (12.05%). Moberg *et al.* [20] suggested an increased incidence of fetal distress in patients with PPROM due to the

loss of protection of umbilical cord that amniotic fluid normally provides. Post-dated pregnancy (>40 weeks gestation) led to non-reassuring fetal heart rate (12.50%) as concluded by James *et al.* [21] in their study. Fetal distress was found in mothers with an abnormal location of placenta (placenta previa) (9.73%) in our study [22]. Fetuses with a true nuchal cord (06.95%) experienced in-utero hypoxia and distress. Begum *et al.* [23] concluded that a true nuchal cord is a sign of fetal distress in their study but did not consider it as an indication for operative delivery. The study done in 2010 by Geidam *et al.* [24] found no significant differences between cases and controls of fetal distress in terms of age, parity, booking status, presence of obstetric conditions, duration of operation and birth weight of babies.

Fetal distress was diagnosed by fetal heart rate (FHR) and presence of meconium. However, an accurate method for establishment of fetal distress is to perform a fetal scalp blood pH estimation which is considered the gold standard for assessment of fetal wellbeing but is not done in our setup. Cardiotocography monitoring is known to overestimate fetal distress [13]. This shows that the method of screening used for making the diagnosis of fetal distress has its own limitations [25]; however, the first response when fetal distress is detected or suspected is that of intrauterine resuscitation which will improve the condition of the fetus and may help to avoid an unnecessary intervention. An alteration of maternal position, Hydration, Oxygen, Intravenous hypertonic dextrose, Amnioinfusion, Tocolysis, etc. [17] are some of the measures which can be used for resuscitation. However, in some cases of fetal distress, an immediate operative delivery may be the only option to ensure a healthy neonate.

Various factors like young age, lack of awareness regarding provision of antenatal care, lack of health education, negligence, financial constraints, environmental & cultural prejudices, male involvement in maternal health care, poor nutritional status of young women (anemia), lack of transport facility, and absence of patient counseling prior to planning of mode of delivery might be responsible for the late booking status with decompensated obstetrics condition resulting in fetal hypoxia, asphyxia and distress [25].

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Effect of Massage Therapy in Cancer Patients

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Abstract

The increase in longevity and incidence of chronic diseases reveals an increased importance in terms of public health. The oncologic illness is a debilitating and progressive pathology with need for prevention and symptomatic relief. In order to find the answer to the question: "What is the effect of massage therapy in cancer patients?" we have reviewed the empirical literature indexed in databases online, finding only 21 articles published between 1990 and 2015. It was possible to verify some of the effects of massage therapy, particularly in relieving pain, decreased anxiety, depression and nausea and increased well-being. However, it was not found the effect of this intervention on the relief of suffering and the quality of life of patients. With the heterogeneity of methodologies, studies suggest the development of more homogeneous research, materials and methods to assess the effects of massage therapy in cancer patients.

Keywords

Patients, Oncology, Massage

1. Introduction

Massage therapy results in touch and its goals are varied including: the ability to help the body relax, feel pleasure to overcome physical problems, releasing emotional blocks, easing of pain, among others.

This type of massage is a method of treatment used in curing a disorder. The massage can be applied for therapeutic purposes, being able to assist in restoring the balance of the various human structures. It is assumed also that its application triggers mechanical effects, painkillers, psychological, structural and thermal effects, which are in line with the main purpose of our report.

According to [1] the purpose of the nurse is to interact with humans in situa-

tions of health or disease, to improve the cultural and social context where they are inserted because they are suffering some kind of transition or anticipating the same. As for the nurse-patient interactions, these can be organized around a main purpose and the nurse uses some therapeutic actions to improve, bring or facilitate the patient's health.

In a second phase, with this report, we can analyse the effect of massage therapy on cancer patients. Being so, we have seen the number of patients with psychological changes increase, including depression, which decreases the quality of life of these patients and the depressive symptoms. This can actually generate a bigger limitation than the cancer itself and can cause suicide in about 50% of the patients. In turn, the anxiety has a prevalence rate of 18% - 35.1% [2].

Among cancer patients, more than 70% suffer from pain caused by disease and/or by handling. Patients in advanced stages of cancer describe the pain as moderate or severe in approximately 40% to 50%, and, according to [3] in 25% - 30% of the patients. In 2004, the Hospice and Palliative Nurse Association has developed a document concerning the pain where it is quoted that this vital sign is one of the most feared by patients in end-of-life. Therefore, it is increased by the physiological stress and decreased by the morbidity, adding the risk of thromboembolism in these patients [4].

The skills of nurses can provide care to a patient in chronic condition aimed at caring for the person with the disease, lessening the suffering, maximizing their well-being, comfort and quality of life [5].

Nurses are crucial elements of a multidisciplinary team as nurses are actively involved in monitoring and pain relief, which requires the screening of the psychological, cognitive and emotional components of pain, including anxiety, depression and grief. Nurses also have previous experience in dealing with the pain, lack a personal, cultural and spiritual influence and can advise the prescription of non-pharmacological interventions in complementarity but not in replacement of pharmacological therapy, knowing their indications, contraindications and side effects [6] [7].

The massage can be curative and preventive, as it can rehabilitate and relief of muscle tension. Patients who benefit from these interventions exhibit lower levels of anxiety and pain, having more control over treatment decisions [8].

Based on the assumption that massage is beneficial to improve the patient's health condition, this literature review has found answers for the question "what is the effect of massage therapy on cancer patients?". Being so, the conclusion is that the purpose of the analysis conducted in this topic was achieved.

2. Methodology

This research was based on a systematic review of the literature with narrative summary. It was held exclusively in online databases, including EBSCO host (Medline, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Psychology and Behavioural Sciences Collection, EJS E-Journals, British Nursing Index), SciELO and RCAAP (open access Scientific

Repository of Portugal). As inclusion criteria we consider articles reported to the period between 1990 and 2015, in full-text, in English and Portuguese and about massage therapy in adult patients with medical diagnosis of cancer. The articles that didn't report the investigation around the theme under study and that did not present the predefined inclusion criteria were deleted.

The research resulted in 120 articles, being 50 articles on EBSCO host, 10 on Scielo and 60 in RCAAP, through the keywords for palliative care, oncology, cancer and massage therapy. Some articles had common databases and after full thorough reading, 21 studies were selected for systematic review. The remaining articles have been removed for not meeting the inclusion criteria (Figure 1).

For an analysis and synthesis of selected articles, it was created a summary table, contemplating various information extracted from each article and then a descriptive analysis of the results.

3. Results

The synopsis of the articles (Table 1) and selected studies are described under paragraph 1, in accordance with the year of publication, study goals, type of study, methods, and results. In relation to the year of publication, it was identified the prevalence of studies published in 2004, 2007 and 2008, with 3 articles each. 2 articles were published in the years 2000, 2003, and 2009, and the rest only obtained a publication for each date mentioned. It was observed that the intervention of massage therapy in oncology nursing professionals offers a limited number of researches, although with some concern over the past fifteen years. Methodologically, it was found that the majority of studies are the quantitative paradigm. The most commonly data collection instruments used was the Visual analogue scale (EVA) for the assessment of symptoms, including pain, anxiety and nausea. It was also observed the use of scale Brief Pain Index (BPI) for the assessment of pain and State-Trait Anxiety Inventory (STAI) for evaluation of anxiety. Only one article used mixed qualitative and quantitative methodology, combining the EVA and the interview as methods. Through the

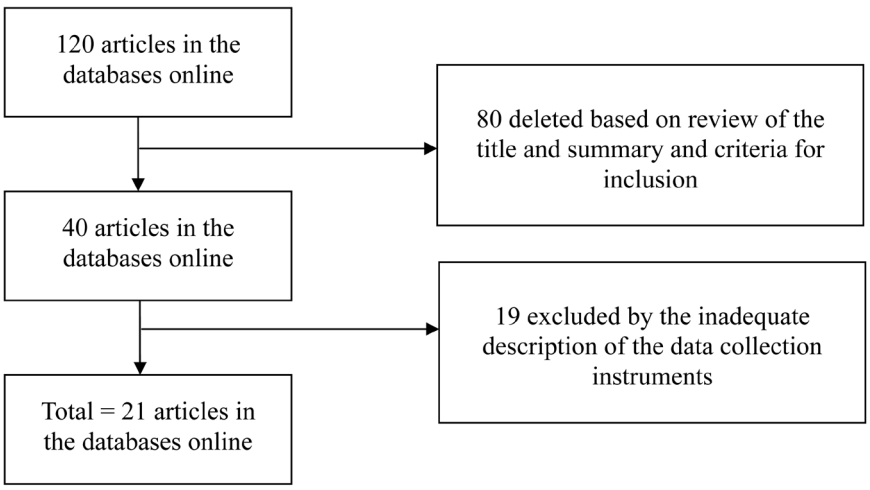

Figure 1. Reduction scheme.

Table 1. General characterization of the articles reviewed.

Author(s)	Date	Goal of the study	Type of study	Methods	Sample	Results
Weinrich, S, & Weinrich, M.	1990	Evaluate the effect of therapeutic massage in the levels of pain	Experimental	Visual Analogue Scale (EVA).	28 cancer patients	-Decrease of immediate pain levels in males and only in the experimental group
Ferrell-Torry, A., & Glick, O.	1993	Evaluate the effectiveness of therapeutic massage in pain, anxiety, vital signs and relaxation	Exploratory	analogue for pain; -Spielberger State Anxiety Inventory; -TA, FC, FR	9 cancer patients	-Reduction of pain in 60% and anxiety X = 24; -Reduction of vital signs, indicating its relaxing action
Ahles, T., Tope, D., Pinkson, B., Walch, S., Hann, D., Whedon, M.	1999	Analyze the impact of therapeutic massage in psychological, physical and Psychophysiological measures	Experimental	State-Trait Anxiety Inventory (STAI), Beck Depression Inventory (BDI), and Brief Profile of Mood States (POMS)	34 patients awaiting bone marrow transplant	It Had immediate effect on stress reduction, nausea and anxiety No significant difference of these variables in the long run
Wilkie, D., Kampbell, J., Cutshall, S., Halabisky, H., Harmon, H., Johnson, L., et al.	2000	Evaluate the effect of therapeutic massage in the levels of pain	Random study	EVA	29 cancer patients	-Decrease in pain intensity at 42% in the experimental group and 25% for the control group
Grealish, L., Lomasney, A., & Whiteman, B.	2000	Analyze the impact of massage therapy in pain, nausea and relaxation	Randomized	EVA.	87 cancer patients	-Immediate Reduction of pain, nausea and relaxation
Toth, M., Kahn, J., Walton, T., Hrbek, A., Eisenberg, D., & Russell, P.	2003	Evaluate the effect of massage therapy in pain and anxiety	Pilot study	Pain, anxiety (VAS) and quality of life (QOL)	26 cancer patients with metastases	-Decreased pain of 5.50 to 3.83 -Increasing the anxiety of 3.83 to 4.75
Post-White, J., Kinney, M. E., Savik, K., Gau, J. B., Wilcox, C., & Lerner, I.	2003	Determine whether the therapeutic massage and touch are more effective than the standard treatment in reducing symptoms of anxiety, mood disorders, pain, nausea and fatigue and increasing relaxation and satisfaction	Randomized	BPI (Brief Pain Index); BNI (Brief Nausea Index); POMS (Profile of Mood States)	164 patients	-Reduced levels of anxiety, pain with decreased use of pain relievers, blood pressure, heart rate and breathing
Soden, K., Vincent, K., & Craske, S.	2004	Evaluate the effectiveness of the massage to decrease pain, anxiety, depression, sleep pattern and improve the quality of life	Randomized	EVA, Verran and Snyder-Halpern (VSH) (scale of sleep), hospital anxiety and depression scale (HAD) and the list of Symptoms Rotterdam (RSCL).	42 cancer patients	No significant differences in terms of improvement in pain management, anxiety or quality of life. -Improved sleeping patterns -Reduction of depression in the massage group
Hernandez-Reif, M., Ironson, G., Field, T., Hurley, J., Katz, G., & Diego, M.	2004	Evaluate the effect of massage therapy in anxiety, mood swings and depression.	Experimental	State Trait Anxiety Inventory (STAI); Profile of Mood States (POMS); symptom Checklist-90-R (SCL-90-R)	34 women with breast cancer	-Minimization in anxiety, depressed mood and anger in the short term; -Minimization, long-term, depression

Continued

Cassileth, B., & Vickers, A.	2004	Evaluate the effect of massage therapy in pain, fatigue, stress/anxiety, nausea and depression.	Almost-experimental	EVA	1290 cancer patients	-Reduction of pain, fatigue, stress, anxiety, nausea and depression approximately 50% and in the same clinic improved 10% more than in the relocation and more durability.
Deng, G., & Cassileth, B.	2005	Evaluate the effect of massage therapy in the relief of symptoms	Prospective	EVA	230 cancer patients	-Decrease in the incidence of muscle fatigue, anxiety, depression, rescue analgesic consumption and improved circulatory and respiratory pattern.
Ferreira, A., & Lauretti, G.	2007	Evaluate the effects of therapeutic massage in the control of pain	Experimental	Pain (EAN) and quality of life (EORTC QLC-C30)	34 cancer patients	-Decrease in consumption of morphine
Wilkinson, S., Love, S., Wesrcombe, A., Gambles, M., Burgess, C., Cargill, A., et al.	2007	Assess the effectiveness of care with massage the management of anxiety and depression	Randomized	subscale of State Anxiety Inventory (SAI), Centre for Epidemiological Studies Depression (CES-D)	288 cancer patients	-There were no significant differences in improvement of anxiety and depression; -The anxiety improved self-report for patients who received massage therapy. This relationship did not exist for the self-report of depression.
Billhut, A., Bergbom, I., & Stenes-Victorin, E.	2007	Evaluate the effect of massage therapy in levels of nausea, anxiety and depression	Randomized	Eva for nausea and for the remaining variables used the HADS	39 women with breast cancer doing chemotherapy	-Significant Reduction of nausea in the experimental group; -There was no differences between anxiety and depression in both groups.
Kutner, S., Smith, M., Corbin, L., Kemphill, l., Benton, K., & Mellis, K.	2008	Evaluate the effectiveness of the massage to decrease pain and distress of symptoms and improve quality of life Evaluate the effectiveness of therapeutic massage in improving the quality of life, pain, stress, suffering	Randomized	Memorial Pain Assessment Card; Brief Pain Inventory [BPI]; McGill Quality of Life Questionnaire; Memorial Symptom Assessment Scale	380 advanced cancer patients	-Immediate improvements in mood and in pain, with more relevance in the experimental group. -In the long term there was no statistical differences corroborate the improvement in quality of life, pain, stress, suffering and in decreasing the use of painkillers.
Currin, J.; Meister. E.	2008	Analyze the impact of massage therapy in pain, physical and emotional desnconforto and fatigue	Not randomized		251 cancer patients	-Decrease of pain, discomfort, emotional and physical fatigue.
Young, C.	2008		Experimental		28 terminal cancer	-Decreased pain and depression for the experimental group.

Continued

Downey, L., Diehr, P., Standish, L., Patrick, D., Kozak, L., Fisher, D., et al.	2009	Evaluate the effect of massage therapy on quality of life and pain	Randomized	MSAS (Memorial Symptom Assessment Scale)	167 patients	-Reduction of pain, however, was not statistically significant
Jane, S. W., Wilkie, D. J., Gallucci, B. B., Beaton, R. D., & Huang, H. Y.	2009	Evaluate the effectiveness of massage in reducing the levels of pain	Quasi-experimental	VAS (anxiety) MSF_MPQ (Short-Form Mc-Grill Pain Questionnaire): BPI (Brief Pain Inventory)	Patients with metastases bone	Immediate effect $p = 0.001$ Medium effect $p < 0.000$ Long effect $p = 0.04$
Adams, R., White, B., & Beckett, C.	2010	Evaluate the effect of massage therapy in the levels of pain	Qualitative and quantitative	EVA -interview	53 cancer patients	-The pain level decreases from 5.18 to 2.33 after the intervention of the massage. -Through the qualitative data describe these illustrate relevant improvements in terms of total pain, emotional well-being, relaxation and sleep patterns.
Sui-Whi, J., Wilkie, D., Gallucci, B., Beaton, R., & Huang, H.-Y.	2011	Evaluate the effectiveness of the massage of pain, anxiety and vital signs	Randomized	(BPI-VAS) pain, anxiety, sleep, relaxation and distress used VAS	36 patients with metastases bone	Effective in the short and in the long term with regard to pain and anxiety, There were no significant effects that could corroborate the changes in heart rate and mean arterial pressure.

analysis of the results of the studies selected, we categorized them according to the focus of nursing—(Classificação Internacional para a Prática de Enfermagem—CIPE), including pain, anxiety, depression, discomfort and suffering.

Taking into account the main results, it can be enhanced that massage therapy reduces: immediate levels of pain in male cancer patients [9], pain levels of approximately 50% plus an improvement of 10% in sick bay rather than in the relocation and more durability of such reduction in pain [10], the intensity of the pain between 60% [11] and 42% [12], as well as its average decrease of 5.50 to 3.83 [13] and 5.18 to 2.33 values [14]. Also, through the qualitative results enhance improvements of total pain [14]. This procedure significantly reduces the levels of pain [15] [16] [17], either immediately [18] [19] [20]; short-term (20/30 minutes) and long term (16/18 hours) [20], although the most significant impact occurred in the first few minutes after the intervention [21]. It also showed a decrease in analgesics used and consumption of rescue analgesics [15] [22], although the consumption of morphine was held for 10 days Nevertheless, there was a reduction in levels of pain after the 5th day [23]. However, there is a study which does not have significant differences that confirm the decline in the use of painkillers [19] [23]. Likewise, it was not shown significant changes in the long term benefits of massage in terms of improvement and pain control [19] [23].

These results corroborate a study, that although there was a decrease of pain, the difference was not statistically significant [22].

As for anxiety, there was a decrease of anxiety with 24% [11] and 50% [10], however only a study anxiety increased from 3.83 to 4.75 [13]. Other studies showed a significant reduction in anxiety and depression after massage therapy [10] [15] [17] [21] [22] [24].

Massage therapy has immediate effect in reducing depression and anxiety and also long-term depression [24] [25]. However, there are studies in which there were no differences in short term between anxiety and depression [26] [27] or that there were no significant differences of depression and anxiety in the long term [27]. Another study confirms these results, noting that there were no significant differences in improvement of anxiety and depression, however, through structured interviews, the self-report of anxiety has improved for patients who received massage therapy. This relationship did not exist for the self-report [28].

As far as the other variables studied, the massage contributes for the immediate reduction of nausea [10] [18] [26] [27], although it was mentioned the non-existence of significant differences of this long-term variable [15] [26]. It was referred the reduction of blood pressure, heart rate and breathing, indicating the relaxing action of massage therapy [11] [15] [22]. The complement of this intervention increases the relaxation [11] [14] [18], because they have immediate effect in depressed mood and anger [19] [24]. This intervention significantly decreased the incidence of muscular fatigue [10] [16] [22], reduces stress [10] and significantly improves the level of emotional well-being, relaxation and sleep patterns). It adds a significant decrease in dimensions of suffering in pain, physical and emotional discomfort, as well as on fatigue after massage [16]. The quality of life significantly improves [23], but in the long term there was no statistically significant differences that could corroborate the improvement of quality of life, stress and suffering [19] [25].

4. Discussion

The results described above emphasize the current need to increase the empirical evidence as well as raise awareness of the benefits associated with this type of interventions that are carried out by nursing staff-therapeutic massage, focusing particularly on the level of patients with oncological pathology.

However, most studies in this systematic review, enhance the effect of massage therapy on decreasing levels of pain and the intensity of some outbreaks that cause discomfort such as: providing relaxation, pleasure, avoid physical problems, release emotional blocks, easing of pain. In this particular case, it is up to the nurse to try to reduce the pain, anxiety, depression and discomfort.

Regarding pain, it was found on the basis of the described studies above, that the results converge because massage therapy decreases pain levels in some very significantly [9]-[18] [20] [21] and in others not so much [19] [22] [23]. We also note that there is the possibility of certain studies support that massage therapy causes patients to reduce the use of painkillers and recourse to SOS [15] [22],

however it was concluded that the differences are not that significant [19] [23].

In terms of anxiety and depression there were also contradictory results because some have revealed positive effects that patients feel in a short term [10] [15] [17] [21] [22] [24], other long-term [24] [25] and others did not reveal any effects [26] [27].

There are also discrepancies about the other areas of nursing, with respect to the effectiveness of massage therapy, the level of nausea, as some studies consider that massage therapy has benefits [10] [18] [26] [27] and others don't mention them [15] [26].

However, it was found that this intervention has obtained positive results, associated with decreased vital signs due to the relaxation promoted [11] [15] [22], in the depressed mood and anger [19] [24], on muscle fatigue [10] [16] [22], in stress [10] and the level of emotional well-being and sleep patterns [14] [25]. There was no statistical differences that could corroborate the improvement in quality of life, stress and suffering [19] [26], justifying the interest of this study.

5. Conclusions

Although the analysis of the results was in many cases contradictory, showing positive and negative or neutral data, the purpose of our study allowed us to conclude that massage therapy has beneficial effects. Being so, in a short term, the level of emotional well-being and relaxation increases, as physical and emotional discomfort, depressed mood, sleep patterns and stress decrease. The level of quality of life and suffering is contradictory, reporting to future investigations continuity perspective.

Through this study, we found the need to step up the research in the field of nursing, broadening the field of nurses, leading to effective therapeutic interventions carried out by these professionals so that we can control and lessen the anxiety and depression.

The nurse has an important role in the patients' recovery, highlighting their feelings and contributing to improve their self-esteem. In this sphere, the massage therapy intervention is essential, as the constant contact of the nurse with the patient encourages the implementation of these actions in order to relieve pain, promote common assistance and improve one's quality of life.

It was noted also that the use of massage therapy is a reality in the universe of human health, nurses increasingly resorting to this type of interventions that had been used for a long time for the patient's comfort. Our role as investigators and researchers is to establish scientifically the effects and benefits of this intervention, in order to add them to the non-pharmacological therapies that are already incorporated in the current health care system.

It is well known that even with the lack of research in this area, the nurses in their day-to-day work already use some therapies for pain control, such as relaxation techniques, cutaneous stimulation (massage, heat/cold, transcutaneous electrical stimulation), among others.

It was found that the relaxation promotes the reduction of these factors, especially the muscle tension, improving the pain, because muscle contraction con-

tributes to exacerbation of pain, focusing on the nerve endings, especially in chronic pain.

With the preparation of this study, we realised that massage therapy decreases total pain levels, both in a short or long term, and that, according to some authors, decreased the use of analgesics and the consumption of painkillers.

It seems that with the implementation of the relaxation massage, it can actually help rebalancing the body. However, most complementary therapies, lead people to submit the relaxation response. Such techniques are related to existing interaction between pain, muscle tension and anxiety, because a patient with pain often presents feelings of apprehension and fear, leading to muscle tension which in turn worsens the pain.

It is important to note that the additional practices that can be used as non-pharmacological treatment of pain, are two groups: techniques or methods carried out by nurses in the nursing consultation and that require expertise or professional qualification.

The care factors depend on the humanistic expectation, associated with the scientific knowledge and concept of mutuality that should exist between the nurse and the patient. That is why the nursing staff have the duty to be aware of the patient' complaints. The body pain that is felt by the patient should always be monitored, so that his story is as improved and real as possible, regarding the evidence of pain and description of its intensity in order to have a combat intervention as efficient as possible.

One of the nurse's contributions is the emphasis on care practice as interpersonal, based on factors that result in the satisfaction of human needs, promoting health, as well as an individual and family growth. It is essential to understand the environment as favouring the personal development and integrated in the biophysical and human behavioural knowledge.

Finally, and still on the basis of the results obtained, we also suggest the development of more research in order to give greater consistency to empirical effects of massage intervention, cancer patients and so contributing to the practice of nursing excellence based on evidence.

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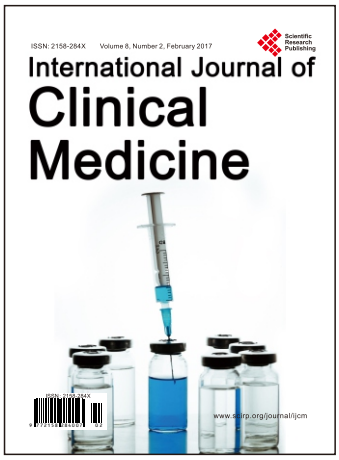

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