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# Nuclear Factor-Kappa B Expression and Acyl-Ghrelin in Egyptian Patients with Non-Alcoholic Fatty Liver

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***Abstract*— BACKGROUND Ghrelin is an inflammation inhibitor hormone. It is secreted from the gastrointestinal tract. It is involved in energy metabolism. Nuclear factor-kappa B (NF-κB) takes part in the initiation and the progression of the cardiovascular and adipose tissue inflammation. The combined role of Ghrelin and NF-κB in non-alcoholic fatty liver disease (NAFLD) pathogenesis is unclear. AIM: To investigate whether acyl-ghrelin level and NF-κB could interplay a role in lipid metabolism and inflammatory injury in NAFLD. PATIENTS AND METHODS: Ninety three adult participates were included in the study, 30 patients had proved nonalcoholic steatohepatitis (NASH), and 38 patients had simple steatosis, as well 25 healthy subjects, as a healthy control group. The control group matches the patients in age, gender and BMI. Full history and clinical examination, abdominal ultrasonography and liver biopsy were done when indicated. Liver function tests, lipid profile, blood sugar, insulin and C- peptide, fasting insulin, and plasma acyl-ghrelin concentrations were measured. Nuclear NF-kB mRNA expression was measured by quantitative RT-PCR. RESULTS: There are significant increase in fasting insulin, insulin C-peptide, HOMA-IR, AST, ALT and γ-GT and a significant decrease in HDL-C was in NAFLD patients compared to control group. In addition, a significant increase in ALT, γ-GT, fasting insulin, insulin C peptide and HOMA-IR were detected in the NASH group compared to group of simple steatosis. The plasma levels of Acyl-ghrelin was significantly decreased in NAFLD groups compared to normal control group, the lowest level was detected in NASH group as compared to group of simple steatosis. There was a significant increase in the expression of NF-kB mRNA in NAFLD groups more than in the normal control group. A significant increase in its level was in NASH than in simple steatosis patients. There was a positive correlation between NF-kB mRNA and BMI, HOMA-IR, ALT, fasting insulin, insulin C-peptide and liver histopathology and acyl-ghrelin was inversely correlated with BMI, HOMR-IR, ALT, fasting insulin, insulin C peptide and liver histopathology. Both were significantly correlated with HDL-C. CONCLUSION: Acyl ghrelin attenuated NAFLD-induced liver injury through down regulation of NF-κB and they are associated with disease progression. Further large scale studies are recommended to consider ghrelin as promising drug for the prevention and treatment of NAFLD.**

***Index Terms*— Acyl-ghrelin; non-alcoholic fatty liver; Nuclear factor-kappa B.**

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## I. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a wide spectrum of diseases affecting the liver with grades of pathology. It causes fat accumulation use of alcohol. Disease spectrum may range from only simple steatosis to cirrhosis. It rarly can lead to hepatocellular carcinoma (HCC) (Bedogni et al., 2006; Vernon et al., 2011).

Oxidative stress and resistance to insulin actions plays a major role initiation and progression of NAFLD (Kawano and Cohen, 2013). The oxidative stress causes injury liver cells with reactive oxygen species (ROS). This leads to hepatocytes inflammation and apoptosis (Natarajan et al., 2014).

Kappa-B (NF-κB) is a nuclear factor that is composed of two subunits, p50 and p65. The two subunits associate with a third protein. IkB- α; is a regulatory protein that inhibits NF-κB. This is done by forming a complex trapping it in the cytoplasm. The NF-κB is expressed in all cell types. It plays a main role as a transcriptional regulator in response to stress of cells (Paschetta et al., 2015).

The pathway NF-κB is linked to oxidative stress and reactive oxygen species (ROS). This is accomplished through the production of inducible nitric oxide synthase (iNOS), cyclooxygenase- (COX-) 2, and metallo-proteinase-9. Hypoxic liver cells release ROS which directly activate hepatic stellate cell. This is through IkB-α phosphorylation and NF-κB signaling activation (Li et al., 2011).

NF-κB takes part in initiation and progression of inflammation. NF-κB up regulates transcription of a wide range of inflammatory mediators leading to signaling pathway activation functions as a proinflammatory (Day, 2006). The NF-κB activation in liver cells and in stellate cells is associated with hepatic insulin resistance, hepatocytes apoptosis, and ultimately the development of NASH and HCC (Robinson and Mann, 2010; Luedde and Schwabe, 2011). A high-fat diet in mice proved to cause lipid accumulation in the liver leading to subacute hepatic inflammation. This occurs via NF-κB activation. It will lead to increased downstream cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β (Cai et al., 2005).

Ghrelin is a peptide, present in the peripheral circulation in two forms: acylated and non-acylated. The stomach and small intestine produce the most amount of circulating

ghrelin. Other organs such as pancreas, lung, kidney and pituitary, and adipocyte tissue produce small amounts of ghrelin (Vanni et al., 2010). Ghrelin is a multifunctional peptide necessary for many biological activities. It caused growth hormone release by its action as an endogenous legend for the growth hormone secretagogue receptor. Furthermore, ghrelin controls energy homeostasis by food intake stimulation and signal transduction to hypothalamic regulatory nuclei and (Chen et al., 2004). In addition, ghrelin is involved in influencing lipid metabolism (Dezaki et al., 2004) and glucose homeostasis by regulating insulin secretion and sensitivity in pancreatic beta-cells (Gauna et al., 2005) and stimulating glucose output by primary hepatocytes (Ikezaki et al., 2002). Furthermore, ghrelin is showing roles in oxigenation and energy regulation (Mao et al., 2015).

Previous studies reported decreased plasma ghrelin levels in several pathological conditions associated with insulin resistance, such as moderate to severe obesity (IR) and type2 diabetes (Tschop et al., 2001; Ikezaki et al., 2002). Ghrelin enhance immune-cell proliferation. It also inhibit secretion of proinflammatory cytokines. Through both mechanisms, inflammatory reactions can be improved (Zhou and Xue, 2009). It is proved that ghrelin administration in rats attenuated NAFLD-induced hepatic injury, inflammation and apoptosis, partly through restoration of LKB1/AMPK and PI3K/Akt pathways (Li et al., 2013). However, the mechanisms of ghrelin-regulated lipid metabolism and the interplay between NF-κB/P65 and ghrelin NAFLD have not been fully elucidated yet, therefore the current study was designed.

## II. The AIM of the Study

The present study was aimed to assess the expression of NFκB/P65 subunit in the peripheral blood of patients with NAFLD and to evaluate their relevance to the circulating levels of ghrelin to find their role in NAFLD pathogenesis, which may be beneficial in the disease amelioration and treatment.

## III. Subjects and Methods

Sixty eight adult patients (31 males and 37 females) were included in the current study, with age ranged from 31-52 years. They were collected from the outpatient Clinic of Hepatology Department, National Liver Institute (NLI) -Menoufiya University. They were proved as NAFLD. The diagnosis of NAFLD was based on abdominal ultrasound, magnetic resonance imaging (MRI) with high hepatic fat fraction (HFF ≥ 5%) (Pacifico et al., 2011), and confirmed by liver biopsy.

Twenty five apparently healthy subjects were included in the study as control group. They were selected from potentially liver transplantation donors. The control group matches the patients in age, gender and BMI.

**Exclusion criteria:**

The following were excluded from the study: other causes of chronic liver disease including: hepatic virus infections (hepatitis A, B, C), other viral hepatitis viruses (cytomegalovirus, and Epstein-Barr virus), autoimmune hepatitis, metabolic liver disease and diabetes mellitus (type 1 or type 2 diabetes), renal disease, alcohol consumption, the use of anti-inflammatory drugs, antibiotics or hepatotoxic dugs.

The research protocol was approved by the local Ethics Committee (NLI-Menoufia University) and a written informed consent was obtained from all subjects participated in the study.

The following was done for the patients and control subjects:

1- Full history and clinical examination including, systolic (Syst. BP) and diastolic blood pressure (Diast. BP).
2- Abdominal Ultrasonography.
3- The body mass index (BMI) was calculated based on the height and body weight: BMI= (kg/m2).
4- Liver biopsy for the patients.

The clinical indication for biopsy was either to assess the presence of NASH and degree of fibrosis. The main histologic features of NAFLD were scored according to the scoring system developed by the NASH Clinical Research Network (CRN) (Kleiner et al., 2005): steatosis [grade 0 (< 5% macrovesicular fat), grade 1 (mild= 5%-33%), grade 2 (moderate = 34%-66%), and grade 3 (severe > 66%)], portal inflammation (0-2), lobular inflammation (0-3), ballooning degeneration (0-2), and fibrosis (stage 0 to 4).

5- The following laboratory investigations were done:

All participates had more than 8 hours overnight fasting before peripheral venous blood samples were drawn, and divided into 3 parts, one part was put on vacutainer plain tube for serum separation and routine investigations, and one part was put on EDTA tube to separate plasma sample and stored at −20°C for estimation of Acyl- Ghrelin. The remaining part was put on heparinized tube to separate mononuclear cells for PCR technique.

Liver enzymes {alanine aminotransferase (ALT), Aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT)}, fasting serum glucose, fasting insulin, lipid profile {total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides}were measured on COBAS 6000(Roche Diagnostics). While insulin and C peptide concentrations were measured on COBAS e 601 module (Electrochemiluminescence Technology, Roche Diagnostics).

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the following equation formula (Emoto, et al, 1999):

Fasting plasma insulin (mU/L) x fasting plasma glucose (mmole/ L)/ 22.5.

HOMA index >3 is a criterion of insulin resistance (Machado and Cortez-Pinto, 2005).

**Determination of plasma Acyl- Ghrelin concentration** (Pradelles et al., 1990):

The Acyl-ghrelin concentrations were measured from fasting plasma samples using a commercial enzyme immunoassay (EIA) kit provided by BioVendor– Laboratorní Medicínaa.s. R & D (Research & Diagnostic products- Czech Republic). Briefly, EIA is based on a double antibody sandwich technique. The wells of the plate supplied are coated with a monoclonal antibody specific to the C-terminal part of Ghrelin. This antibody will bind to any Ghrelin introduced into the wells (standard or sample). The acetyl cholinesterase (AChE)- Fab' conjugate (Tracer) which recognizes the N-terminal part of Acylated Ghrelin is also

added to the wells. The two antibodies then form a sandwich by binding on different parts of the Acylated Ghrelin. The sandwich is immobilized on the plate so reagents in excess may be washed away. The concentration of Acylated Ghrelin is determined by measuring the enzymatic activity of immobilized Tracer using Substrate Solution (Ellman's Reagent). AChE Tracer acts on Ellman's Reagent to form a yellow compound that strongly absorbs at 414 nm.The intensity of the color, which is determined by spectrophotometry, is proportional to the amount of Acylated Ghrelin present in the well during the immunological incubation.

The intra-assay and inter-assay variations of coefficients (CV) of less than 5% and 14%, respectively, sensitivity limit of sensitivity: < 5 pg/mL, and Limit of detection in the sample before dilution < 8 pg/mL.

**RNA extraction and reverse-transcription of NF- κB p65 mRNA expression:**

Heparinized blood was used to isolate mononuclear cells (PBMC) by using sedimentation gradient by Ficoll–Hypaque and centrifugation for 30 minutes at 400g, at room temperature then washed with phosphate buffered saline (PBS). The PBMCs samples were stored at- 80°C until total RNA extraction. The DNA was extracted from 200 µL whole peripheral blood using Qiagen RNeasy Mini Kit according to the manufacturer's instructions (QIAGEN, Inc., Hilden, Germany). RNA was eluted and its concentration was measured by using spectrophotometer at wave length 280 nm. The reverse transcription kit ReverTra Ace quantitative PCR RT Kit (Japan) was used to synthetize complementary DNA (cDNA) according to the manufacturer's instruction.

Real-time PCR was done using ABI TaqMan kit (Applied Biosystems, Carlsbad, CA) and the ABI 7500HT Real Time PCR System (Applied Biosystems, USA). The primers used for NF-kB p65 (Yi et al., 2014) were; forward:59-ATCCCATCTTTGACAATCGTGC-39,reverse: 59-CTGGTCCCGTGAAATACACCTC-39, and amplification Product is 153 bp.

The primers for GAPDH were: forward: 59-GCACCGTCAAGGCTGAGAAC-39, reverse: 59-TGGTGAAGACGCCAGTGGA-39, and amplification product is 138 bp.

The cycles for PCR reaction were performed as follows: pre-denaturation at 95°C for 2 minutes, followed by denaturation at 95°C for 10 second, annealing at 58°C for 20 seconds, and ended by extension at 72°C for 30 second, with 40cycles in total, then followed by extension at 72°C for 5 min. The melting curve was used and the relative amounts of NF- κB and GAPDH mRNA were expressed as ΔCT (ΔCT=CT value of the target gene - CT value of internal control).

## IV. STATISTICAL METHODS

The data were collected and statistically analyzed using SPSS computer program version 21. The data were expressed as mean ± SD and differences between 2 groups were analyzed by student t test for parametric variable distribution or Mann-Whitney test for non parametric. One way analysis of variance (ANOVA) F-test was used for comparison between more than 2 groups. Pearson's correlation coefficient was used to test the relationship between various variables. P value is considered significant if <0.05.

## V. RESULTS

No significant differences between NAFLD and controls regarding age, systolic and diastolic blood pressure, BMI, Fasting and post prandial blood sugar, HbA1c, serum albumin, triglyceride, total cholesterol and LDL-C (p>0.05). On the contrary, fasting insulin, insulin C peptide, HOMA-IR, AST, ALT and γ-GT were significantly increased (p<0.001, p<0.001, p<0.001, p<0.05, p<0.05, p<0.05 respectively), and HDL-C was significantly decreased in NAFLD group compared to control group (p <0.05) (**Table 1**).

**Table (1) Comparison between NAFLD and control groups as regard clinical and routine laboratory data.**

| Parameters | NAFLD (no=68) M±SD | Controls (n=25) M±SD | P-value |
|---|---|---|---|
| **Age (years)** | 39.4±9.2 | 36.4±8.3 | >0.05 |
| **Syst. BP (mmHg)** | 122.3±9.2 | 117.2±8.6 | >0.05 |
| **Diast. BP (mmHg)** | 85.1±7.3 | 81.4±7.5 | >0.05 |
| **BMI (kg/m2)** | 27.9±2.71 | 25.8±3.11 | >0.05 |
| **Fasting glucose (mg/dL)** | 92.6± 7.2 | 87.5±5.4 | >0.05 |
| **2-h glucose (mg/dL)** | 110.4±8.7 | 102.8±6.7 | >0.05 |
| **HbA1c (%)** | 5.2± 0.8 | 4.6± 0.5 | >0.05 |
| **Fasting Insulin (mU/L)** | 15.2±4.7 | 7.9±1.8 | <0.001 |
| **Insulin C peptide ( pmol/L)** | 1022±147 | 685±91 | < 0.001 |
| **HOMA-IR** | 6.05 ± 1.21 | 2.48 ± 0.46 | <0.001 |
| **AST (U/L)** | 51.1±10.6 | 24.2±6.5 | <0.05 |
| **ALT (U/L)** | 57.4 ± 21.5 | 23.6 ± 7.4 | < 0.05 |
| **γ-GT (U/L)** | 29.3 ±5.9 | 17.6±4.2 | < 0.05 |
| **S. Albumin (g/dL)** | 4.02± 0.37 | 4.27± 0.48 | >0.05 |
| **Triglycerides (mg/dl)** | 106.3±11.7 | 79.6±8.4 | >0.05 |
| **Total cholesterol (mg/dl)** | 174.2±16.2 | 162.4±15.5 | >0.05 |
| **HDL-C (mg/dl)** | 32.5±6.4 | 49.1± 7.2 | <0.5 |
| **LDL-C (mg/dl)** | 123.1±19.2 | 104.7 ±11.5 | >0.05 |

As comparing between 2 NAFLD subgroups showed a significant increase in ALT, γ-GT, fasting insulin, insulin C peptide and HOMA-IR (p<0.05 for each) were detected in the NASH group compared to group of simple steatosis. In contrast, age, BMI, AST, fasting and postprandial blood sugar, triglyceride, total cholesterol and LDL-C showed no significant differences between two subgroups (p>0.05) (Table 2).

Histopathology of 68 patients revealed that, 30 patients had proved nonalcoholic steatohepatitis (NASH) {11(%) were mild steatosis= grade 1, 16 (%) were moderate steatosis=2, 3(%) cases were severe=grade 3 steatosis)}, and remaining 38 (100%) patients had simple steatosis (7 of them were mild= grade 1, and 31 were minimal steatosis=grade 0) (**Table 2**).

**Table (2) Comparison between NAFLD subgroups regarding routine parameters.**

| Parameters | NASH group (no=30) M±SD | Simple steatosis (n=38) M±SD | P-value |
|---|---|---|---|
| **Age (years)** | 51.3±5.8 | 47.5±6.8 | >0.05 |
| **BMI (kg/m2)** | 28.7±2.2 | 27.4±2.5 | >0.05 |
| **AST (U/L)** | 56.4±6.2 | 46.3±7.1 | >0.05 |
| **ALT (U/L)** | 68.3 ±7.9 | 44.6±5.1 | <0.05 |
| **γ-GT (U/L)** | 32.8±3.1 | 21.6±2.4 | <0.05 |
| **Fasting glucose (mg/dL)** | 94.1±8.7 | 80.3 ±5.1 | >0.05 |
| **2-h glucose (mg/dL)** | 114.2±5.9 | 106.1±4.3 | >0.05 |
| **Fasting Insulin (mU/L)** | 18.9±2.1 | 12.7±3.2 | <0.05 |
| **Insulin C peptide (pmol/L)** | 1096±77 | 823±52 | <0.05 |
| **HOMA-IR** | 6.89 ± 0.37 | 5.22±0.74 | <0.05 |
| **Triglycerides (mg/dl)** | 110.1±9.2 | 99.4±4.7 | >0.05 |
| **Total cholesterol (mg/dl)** | 185.6±10.2 | 178.6±9.1 | >0.05 |
| **HDL-C (mg/dl)** | 36.5±4.3 | 31.4±5.2 | >0.5 |
| **LDL-C (mg/dl)** | 132.1±9.2 | 128.1±7.8 | >0.05 |
| **Grade of steatosis:**<br>Grade zero<br>Grade 1<br>Grade 2<br>Grade 3 | <br>0 (0%)<br>11 (36.7%)<br>16 (53.3%)<br>3 (10%) | <br>31 (81.6%)<br>7 (18.4%) | <br><br>< 0.001 |

The plasma levels of acyl-Ghrelin was significantly decreased in NAFLD groups compared to normal control group (p1<0.001), its level more significantly decreased when compared NASH to normal controls (p2<0.0001) or simple steatosis to controls (p3<0.01). The lowest level was detected in NASH group as compared to group of simple steatosis (p<0.05) (Table 3 & Figure 1).

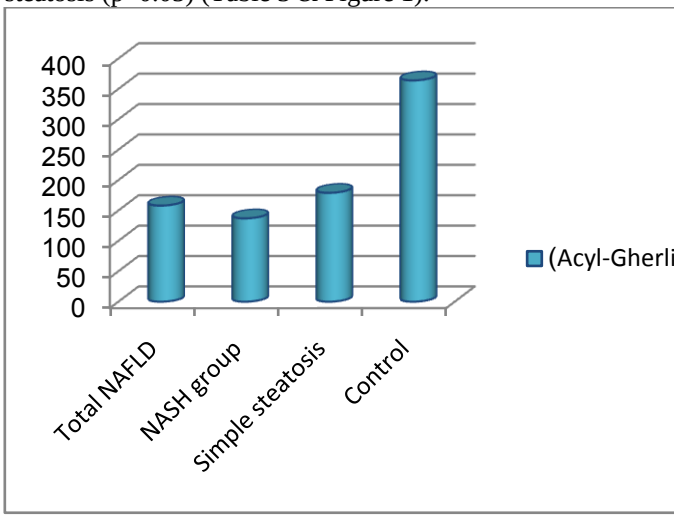


**Fig. (1) The plasma levels of Acyl-Ghrelin among the studied groups.**

Real-time quantitative RT-PCR showed that the expression of NF- κB p65 mRNA was significantly increased in NAFLD groups compared to normal control group (p1<0.01). Additionally, its level was significantly increased compared NASH or simple steatosis to normal controls (p2<0.001 and p3<0.05 respectively) or compared simple steatosis to controls (p4<0.05) (Table 3 & Figure 2).

**Table (3) Comparison between the studied groups regards NF- κB mRNA and plasma Acyl-Ghrelin.**

| Parameters | Total NAFLD (no=68) M±SD | NASH group (no=30) M±SD | Simple steatosis (n=38) M±SD | Controls (n=25) M±SD |
|---|---|---|---|---|
| **Acyl-Ghrelin (pg/ml)** | 158± 45 | 137± 31 | 179± 28 | 364± 78 |
| **P value Post-Hoc** | P1<0.001, P2<0.0001<br>P3<0.01, P4<0.05 | | | |
| **NF-κB p65 mRNA level** | 0.078±0.016 | 0.089±0.005 | 0.066±0.004 | 0.021±0.003 |
| **P value Post-Hoc** | P1<0.01, P2<0.001<br>P3<0.05, P4<0.05 | | | |

P1<0.05= comparison between total NAFLD group and controls, p2<0.001= between NASH and controls, p3= between simple steatosis and controls, p4= between NASH and simple steatosis.

The correlation analysis indicated that NF- κB p65 mRNA was positively correlated with BMI(r =0.249, p<0.05), HOMA-IR (r= 0.249, p<0.01), ALT (r= 0.275, p<0.05), fasting insulin (r= 0.278, p<0.05), insulin C-peptide (r= 0.291p<0.05) and liver histopathology (r= 0.420, p<0.01) and negatively correlated with HDL-C (r = -0.362, p<0.01) and acyl- Ghrelin plasma levels (r= -0.344 p<0.01). There was no correlation between NF- κB p65 mRNA and age, sex, triglyceride or total cholesterol.

Plasma acyl-ghrelin was inversely correlated with BMI, HOMR-IR, ALT, fasting insulin, insulin C peptide and liver histopathology. It was significantly correlated with HDL-C. While no significant correlation was detected between acyl-ghrelin and each of age, sex, triglyceride and total cholesterol (Table 4).

**Table (4) Correlation study between NF- κB mRNA and plasma Acyl- Ghrelin to risk factors in NAFLD patients (n=68).**

| Parameters | NF- κB 65 mRNA | | Acyl- Ghrelin | |
|---|---|---|---|---|
| | r | P | R | P |
| **Age** | 0.162 | >0.05 | 0.031 | >0.05 |
| **Sex** | 0.086 | >0.05 | 0.115 | >0.05 |
| **BMI** | 0.249 | <0.05 | - 0.264 | <0.05 |
| **HOMA-IR** | 0.398 | <0.01 | - 0.286 | <0.05 |
| **ALT** | 0.275 | <0.05 | -0.352 | <0.01 |
| **HDL-C** | -0.362 | <0.01 | 0.248 | <0.05 |
| **Triglyceride** | 0.131 | >0.05 | -0.032 | >0.05 |
| **Total cholesterol** | 0.059 | >0.05 | 0.126 | >0.05 |
| **Fasting Insulin** | 0.278 | <0.05 | -0.291 | <0.05 |
| **Insulin C peptide** | 0.291 | <0.05 | -0.274 | <0.05 |
| **Liver histopathology** | 0.420 | <0.01 | -0.286 | <0.05 |
| **Acyl-Ghrelin** | -0.344 | <0.01 | ---------- | ---------- |

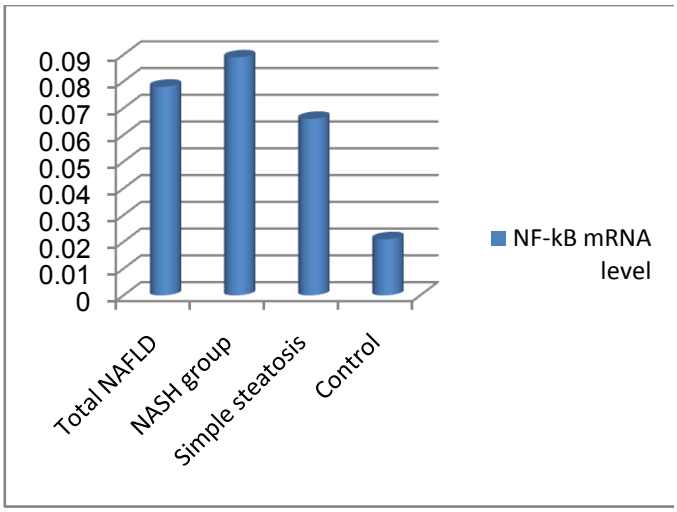


**Figure (2) The levels of NF-κB p65 mRNA among the studied groups.**

## VI. Discussion

Whereas over-nutrition and obesity are the main cause of simple fatty liver, it is still unclear why 10% of affected individuals are developing NASH. NAFLD has emerged as a major health problem throughout the world. Soluble mediators synthesized by immune system cells (e.g. cytokines/ chemokines) and adipose tissue are involved in NAFLD and in the regulation of insulin action (Angulo, 2013).

However, Zhou and Xue, (2009) study reported that ghrelin improved inflammatory reactions through immune-cell proliferation stimulation. It also inhibits proinflammatory cytokines secretion. However other report detected that ghrelin administered to rats resulted in attenuation of NAFLD-induced hepatic injury, inflammation and apoptosis (Li et al., 2013). we designed this study to clear this conflict.

In the current study, a significant increase in the fasting insulin, insulin C-peptide, HOMA-IR, AST, ALT and γ-GT were detected in NAFLD group compared to control group while a significant decrease in HDL-C in NAFLD group compared to control group, suggesting the prominent characters of NAFLD group. The laboratory findings most closely associated with the presence of NAFLD in a large series of patients were insulin resistance and hyperinsulinemia, even in lean subjects with normal glucose tolerance (Bedogni et al., 2005). While, no significant differences was detected regarding age, systolic and diastolic blood pressure, BMI, fasting and post prandial blood sugar, HbA1c, serum albumin, triglyceride, total cholesterol and LDL-C suggesting matched group with adjustment of other risk factors in all participates and hypertension and especially systolic hypertension is also an independent predictor of NAFLD (Dixon et al., 2001).

As comparing between two NAFLD subgroups showed a significant increase in ALT, γ-GT, fasting insulin, insulin C-peptide and HOMA-IR were detected in the NASH group compared to group of simple steatosis. In contrast, age, BMI, AST, fasting and postprandial blood sugar, triglyceride, total cholesterol and LDL-C showed no significant differences between two subgroups. At a study by Pathik et al. (2015), they found that NAFLD patients with raised transaminases have significant liver inflammation and fibrosis. BMI, abdominal girth, triglyceride levels, cholesterol levels were higher in patients with advanced stage of liver fibrosis

In a study by Fracanzani et al. (2008), they reported that the aminotransferase levels may be elevated two to four times over the upper limit of normal in NAFLD/NASH, with ALT being higher than AST, in contrast to alcoholic steatohepatitis. However, in mild liver affection, routine liver function tests are normal or shows only mild elevations in aminotransferase levels. Alkaline Phosphatase and γ-GT show also mild elevations from 1.5-3 times the upper limit of normal (Manuela et al., 2014).

This study clearly showed that ghrelin levels are reduced in NAFLD patients. The plasma level of Acyl-ghrelin was significantly decreased in NAFLD groups compared to normal control group; its level more significantly decreased when compared NASH to normal controls or simple steatosis to controls. The lowest level was detected in NASH group as compared to the group of simple steatosis. Pinkney and Williams, (2002) reported that, ghrelin stimulated the intake of food and reduced levels may be teleological aiming to prevent the increase in body weight. This is not applicable in

NAFLD patients as ghrelin is not related totally to BMI. Inverse relationship between Ghrelin and insulin was there, however but HOMA-IR values were the most significant predictor of low ghrelin concentrations. Based on this it appears that ghrelin secretion may be in part under the control of insulin or, more likely, under insulin resistance control. This might be through undefined circulating factors. We had tried to find a link between hormonal levels with liver function parameters as a potential factors causing low ghrelin levels. However there were negative results.

Tacke et al. (2003) demonstrated normal ghrelin levels in non cirrhotic patients and slightly elevated levels in cirrhosis. In their study, it was noted that Ghrelin level was only related to the severity of the disease. Patients in their study included only advanced disease of the liver being evaluated for liver transplantation. This correlation, might not be correct as it may be due to the anorexia and decreased food intake noted usually in such group of patients.

Real-time quantitative RT-PCR showed that the expression of NF-κB p65 mRNA was significantly increased in NAFLD groups compared to normal control group. Additionally, its level was significantly increased in NASH or simple steatosis compared to normal controls. Mao et al, (2015) reported lipotoxicity attenuation by ghrelin. This was through autophagy stimulation and NF-κB inhibition. The study was conducted in mice. The therapeutic effect of ghrelin on NAFLD was previously investigated by Manuela et al. (2014) and the results indicate that ghrelin attenuates lipotoxicity by autophagy stimulation and NF-κB inhibition. This provides evidence that autophagy deficiency leads to steatosis, since autophagy digests lipid droplets, and drugs that increase autophagy may be a potential therapeutic approach in NAFLD.

NF-κB activation in the cytosol is regulated by inhibitory protein, IkB. The degradation of IkB allows nuclear translocation of NF-κB. In the nucleus it binds to promoter sites for gene transcription (Feng et al, 2014). The activation of NF-κB genes regulated plays important and conserved roles in immune and stress responses, and impact processes such as apoptosis, proliferation, differentiation, and development (Oeckinghaus and Ghosh, 2016).

In the current study, histopathology of 68 patients revealed that 30 patients had proved NASH which about 44% of patients, and remaining 38 patients had simple steatosis. Approximately 30% to 40% of patients with NAFLD develop NASH. It is estimated that 10% to 30% of patients with NAFLD develop cirrhosis after 10 years, with NAFLD believed to be the most common cause of cryptogenic cirrhosis. A diet rich in saturated fats and refined carbohydrates leads to hyperinsulinemia and fatty liver. Dietary intervention remains the current standard of care for NAFLD and NASH; however, this intervention often fails to control the disease (Manuela et al., 2014).

The correlation analysis in our study revealed that NF- κB p65 was positively correlated with BMI, HOMA-IR, ALT, fasting insulin, insulin C-peptide and liver histopathology and inversely correlated with acyl-ghrelin plasma levels

Plasma acyl-ghrelin was inversely correlated with BMI, HOMR-IR, ALT, fasting insulin, insulin C-peptide and liver histopathology.

There was an inverse relationship between insulin and Ghrelin values. However HOMA-IR values were the most significant predictor of low ghrelin levels. Accordingly, ghrelin secretion may be controlled by insulin or, more likely, by insulin resistance, through undefined circulating factors (Manuela et al., 2014). This conclusion is also supported by a study of Maher et al., in 2008 who demonstrated that insulin resistance stimulates phosphorylation. Subsequently degradation of IKB, accompanied by translocation of activated NF-κB to the nucleus. This will cause inflammatory cascades, that will aggravate NF-κB activation.

Mykhalchyshyn et al. in 2015, has demonstrated that elevated Acyl-ghrelin level was associated with NAFLD. Patients with elevated transaminases had significantly higher Acyl-ghrelin levels. Therefore an increase of Acyl-ghrelin can be used as a diagnostic marker for NAFLD detection in patients with type 2 diabetes. In the short term, ghrelin may reduce NAFLD-induced liver injury and reduce lipid accumulation. In long-term effect in fibrogenic cells, ghrelin may worsen chronic liver disease (Mao et al., 2015).

Another study demonstrated that hepatic fibrosis reductions in rodents by ghrelin. This was through attenuation of hepatocytes injury (Moreno et al., 2010).

## VII. CONCLUSION

Our findings demonstrate that the decreased levels of plasma ghrelin and over expression of its regulator (NF-κB) may induce liver injury and enhance NAFLD progression. Large-scale studies are required to develop drugs for the prevention and treatment of NAFLD, aiming at the NF-κB inhibition and ghrelin stimulation.

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