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Regulation of Transport of Ca^{2+} NMDA-Receptors in Rat Brain Synaptosomes Under the Influence of Polyphenols

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Abstract

It is known that disturbance of Ca^{2+} dynamics in nerve cells is universally involved in all pathologies of Alzheimer's disease (AD). It is likely that the use of chemical agents or small molecules specific for Ca^{2+} channels or membranes of intracellular organelles to correct Ca^{2+} dysregulation of neurons may open up a new approach to the prevention and treatment of AD. The experiments were carried out according to the Weiler method. Synaptosomes were isolated from the brain of rats by two-stage centrifugation at a temperature of 4°C.To measure the amount of cytosolic Ca^{2+} synaptosomes were calculated by the Grinkevich equation. The results of the experiments show that there is a possibility of competition between different polyphenols of Caralinia, Granatuum and glutamate for the site of regulation of the opening ion channels of NMDA-receptors.

Keywords: synaptosome, NMDA receptors, glutamate, calcium.

1. Introduction

In recent years, there has been a significant increase in the number of diseases of the nervous system, which, due to their prevalence and consequences, are classified as socially significant neurodegenerative diseases due to a violation of calcium homeostasis of excitable nerve cells in the brain. Alzheimer's disease (AD) is the most common type of neurodegenerative disease and is characterized by memory loss and cognitive decline, which eventually leads to dementia (Alzheimer's Association, 2017). Although the etiology of AD has not been determined, the pathological features associated with AD are mainly the accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau (Tiraboschi et al., 2004). More than 30 million people worldwide have developed asthma (Karch et al., 2014). Cellular Ca^{2+} homeostasis plays a key regulatory role in many aspects of neuron physiology, including growth and differentiation, action potential properties, synaptic plasticity, learning, and memory. Impaired cellular Ca^{2+} also contributes to pathophysiological conditions

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such as necrosis, apoptosis, autophagy deficiency, and degeneration (Bezprozvanny, 2009). The hypothesis that Ca^{2+} dysregulation leads to neurodegeneration was first proposed by Dr. Khachaturian in the mid-80s. He suggested that a persistent imbalance of cellular Ca^{2+} could impair normal neuronal function and ultimately lead to neurodegenerative diseases, including AD (Khachaturian, 1987). Although various types of Ca^{2+} disorders have been reported in AD cells for over 30 years (Peterson et al., 1985), both amyloid and tau hypotheses have been described as the main causative factors in AD. Drugs targeting Aβ have been largely unsuccessful, and Ca^{2+} disruption may have occurred before plaque and tangle deposition. Thus, drugs aimed at correcting the Ca^{2+} abnormality may provide an alternative therapeutic strategy for AD. In this review, we will consider the mechanisms involved in Ca^{2+} disorders in AD and propose an understanding of the correction of Ca^{2+} disorders as a therapeutic strategy for the prevention and treatment of AD.

NMDA-receptors (NMDAR) are Ca^{2+}-favoring glutamate-gated ion channels that are expressed in most central neurons and were initially held responsible for neuronal injury, owing to their high Ca^{2+} permeability and conductance properties (Rothstein,.1996). Continuous activation of large numbers of NMDAR (especially the NR1/NR2B-subtype) leads to increases in intracellular calcium loads and catabolic enzyme activities, which can trigger a cascade of events eventually leading to apoptosis or necrosis (Ndountse, Chan 2009). These downstream effects include mitochondrial membrane depolarization, caspase activation, production of toxic oxygen and nitrogen free radicals, and cellular toxicity (Jung et al., 2009; Fan et al., 2007). The NMDAR are also effective in mediating excitotoxic neuronal injury. For example, neurons expressing high levels of NMDAR are lost early in the striatum of individuals affected with neurodegenerative disease, and injection of NMDAR agonists into the striatum of rodents or non-human primates recapitulates the pattern of neuronal damage in AD (Handley et al., 2006).

It has been proven that the T-type channel can contribute to exocytosis and may be involved in spontaneous synaptic transmission. L-glutamate is the main excitatory neurotransmitter in the brain that stimulates the receptor-gated Ca^{2+} channel. Depending on the mode of operation, L-glutamate activates two classes of receptors, namely the ionotropic receptor (iGluR) and the metabotropic receptor (mGluR). MGluRs are associated with G proteins that generate Ca^{2+} signals by activating phospholipase C or by modulating adenylate cyclase activity. While α-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid sensitive receptor (AMPAR) and NMDAR are the two main types of iGluR. Both AMPAR and NMDAR are important for neuronal plasticity by modulating long-term potentiation (LTP) of postsynaptic neurons. NMDAR is permeable to both Na^{+} and Ca^{2+}. NMDAR-mediated Na^{+} influx promotes postsynaptic depolarization, while Ca^{2+} entering through NMDAR generates Ca^{2+} transients that promote neuronal transcription upon LTP induction (Miyashita et al., 2012). Like iGluRs, ionotropic purinergic receptors mediate extracellular Ca^{2+} influx in response to extracellular ATP. They are the main postsynaptic Ca^{2+} entry channels at the resting potential when NMDARs are blocked by $Mg^{2+,}$ and their activation has multiple modulating effects on synaptic plasticity (Khoshimov et al., 2020; Pankratov et al., 2003).

Several therapeutic agents currently targeting plasma Ca^{2+} channels have shown good efficacy in both in vitro and *in vivo* models of AD. Some of them are already approved drugs for the treatment of AD (memantine) or are undergoing clinical trials (nimodipine) (Zhang et al., 2016). However, all of these drugs target plasma membrane channels rather than the most common disrupted intracellular Ca^{2+} signaling pathways, such as the ER and lysosome pathways. Therefore, additional research efforts are needed to elucidate the role of intracellular Ca^{2+} in the pathogenesis of AD. As our knowledge of the molecular mechanisms linking Ca^{2+} disturbances to AD pathology becomes clearer, more specific therapeutic agents targeting channels on the membranes of intracellular organelles such as the ER, mitochondria, and lysosomes will be developed and provide new hope for prevention and treatment. Collected all these literature data shows that, in Alzheimer's disease, there is a hypothesis of excitotoxicity of glutamate (Wen et al., 2016).

It is known that various mechanisms associated with glutamate excitotoxicity are explained by neuronal death characteristic of various neurodegenerative diseases, including an increase in intracellular calcium levels, accumulation of oxidizing free radicals, impaired mitochondrial function, and activation of apoptosis and autophagy programs. The identification of a key post-receptor signaling event that leads to neuronal dysregulation and death has the potential to become a molecular target for therapeutic interventions in various neurodegenerative diseases.

(Khoshimov et al., 2018). Based on this data, the study focuses on the mechanism of action of polyphenolic compounds on glutamate-binding sites of NMDAR in rat brain synaptosomes.

The aim of the study is to characterize the mechanism of action of polyphenolic compounds of Caralinia isolated from *Caralinia kaspia* plants and Granatuum polyphenol isolated from *Punica granatum* plants *in vitro* on the regulation of Ca^{2+} NMDA glutamate receptor transport in rat brain synaptosomes.

2. Materials and methods

2.1. Isolation of synaptosomes

Experiments were carried out on 20 outbred albino male rats weighing (280-300 g) kept in the standard diet of the vivarium. All experiments were carried out in accordance with the requirements of the World Society for the Protection of Animals and European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (European Convention..., 1986). Synaptosomes are obtained by two-stage centrifugation Centrifuge K-24 (ELN13893354.Veb MLV Zenrifugenbau Engelsdorf. Germany) (Weiler et al., 1981). The entire isolation procedure is carried out at -4°C. After decapitation, the brain is removed as quickly as possible and crushed on ice. The crushed tissue is homogenized at a ratio of 1:10 in the isolation medium - 0.32 M sucrose solution in 0.01 M Tris-HCl buffer with the addition of 0.5 mM EDTA (pH 7.4). The obtained homogenate is exposed to a 4-stage centrifugation. The supernatant after the first centrifugation (10 min, 4500 rpm) is carefully removed without capturing the myelin layer and exposed to further centrifugation for 20 min at 14000 rpm. The obtained dense precipitate P2 is resuspended in the isolation medium. The obtained suspension is used further in the experiment as a coarse synaptosomal fraction (synaptosomal-mitochondrial). In the case of 4-stage isolation, the second centrifugation is carried out at 11,000 rpm for 20 minutes. The dense pellet of P2 is resuspended in 0.32 M sucrose solution (pH 7.4) and then carefully layered on 0.8 M sucrose solution (pH 8.0), after which it is centrifuged for 25 minutes at 11,000 rpm. As a result of centrifugation in a sucrose gradient, factions are separated - mitochondria settle tightly at the bottom of the tube, and synaptosomes remain in suspension in a layer of 0.8 M sucrose. This layer is carefully removed, mixed with an equal amount of isolation medium and left for 15 minutes to restore the ultrastructure of synaptosomal particles, after which it is exposed to further centrifugation at 14,000 rpm for 30 minutes. The dense final precipitate P4 is resuspended in the isolation medium and then used in the experiment as a synaptosomal faction.

2.2. Measuring intracellular Ca^{2+}

To measure the amount of membrane bound Ca^{2+}, 20 μM chlortetracycline (CTC) was added to synaptosomes placed in a medium similar to that used for cell isolation, but without apyrase and $MgCl_2$. It is incubated 60 min. to achieve maximum interaction of CTC with membrane-bound Ca^{2+}, both on plasma and intracellular membranes. The CTC excitation wavelength is 405 nm, the registration wavelength is 530 nm. The results were expressed as percentages, taking as 100 % the difference between the maximum fluorescence intensity (fluorescence of the dye saturated with Ca^{2+}) and its minimum value (fluorescence of the indicator in the absence of Ca^{2+}) obtained after the addition of EGTA.

The amount of cytosolic Ca^{2+} ($[Ca^{2+}]_{in}$) was calculated using the Grinkevich equation (Grynkiewicz et al., 1985) in synaptosomes isolated from rat brains. To measure free cytosolic Ca^{2+}, synaptosomes ($1x10^8$ cells/ml) were loaded with 4 μM Fura-2AM acetoxymethyl ester for 40 min at 37°C. At the same time, in the dye molecules that have penetrated into the cytoplasm, under the action of intracellular esterases, the ester group is cleaved off, resulting in the Fura-2 anion that binds Ca^{2+}. After completion of the loading, the dye remaining in the medium was removed by double washing and centrifugation in standard medium. In the experiments, the cell concentration in the cell was $5x10^6$ cells/ml. Fluorescence excitation was induced at 337 nm and fluorescence registration at 496 nm. Ca^{2+} saturated dye fluorescence (Fmax) was determined by adding 50 μM digitonin to cells loaded with Fura-2AM. Fmin was determined by measuring the fluorescence intensity in a calcium-free medium, Fmin = $[(Fmax - Faf)/3]^+$ Faf, where Faf is cell autofluorescence determined by adding 0.1 mM $MnCl_2$ to thymocytes loaded with Fura-2AM and processed with digitonin.

2.3. Statistical analysis

The measurements were carried out on a universal spectrometer USB-2000 (USB2E7916.OceanOptics.USA.2010). Statistical significance of differences between control and experimental values, determined for a data series using a paired t-test, where control and experimental values are taken together, and an unpaired t-test, when taken separately. A P value <0.05 indicates a statistically significant difference. The results obtained are statistically processed in Origin 7.5 (Origin Lab Corporation, USA).

3. Results and discussion

Many neurodegenerative diseases are accompanied by an increase in glutamatergic transmission, which occurs due to an increase in the release of L-glutamate. The excitatory neurotransmitter L-glutamate causes damage and death of neurons, and therefore the damaging effect of glutamate on neurons is referred to as "excitatory amino acid toxicity" or “excitotoxicity” (Duchen, 1999; Khoshimov et al., 2021; Rakhimov et al., 2021, Khoshimov et al., 2021).

The experiments used synaptosomes, which are an adequate and convenient model for studying presynaptic processes. The activity of L-glutamate was judged by the change in the intensity of the fluorescent signal, by the change in $[Ca^{2+}]_{in.}$

Polyphenols are known to be a group of naturally occurring phytochemicals that are present in high amounts in fruits, vegetables and natural foods and are characterized by the presence of several hydroxyl groups on aromatic rings. These compounds are divided into two main categories: the flavonoids and non flavonoids, based on the number of phenol rings and the way in which these rings interact.

The experiments were performed in two stages both in the presence and in the absence of physiological Ca^{2+} concentrations. In experiments, the effect of L-glutamate on the level of $[Ca^{2+}]_{in}$ synaptosomes from the rat brain was investigated. The fluorescence ratio was determined using a Ca^{2+}-sensitive chlortetracycline (CTC) probe. Ethylene glycol bis[2-ethylamino] tetraacetate (EGTA) was used to determine the removal of Ca^{2+} from the extracellular medium.

The chelating ability of L-glutamate with respect to calcium ions was studied in intact synaptosomes both in the presence and in the absence of EGTA. As a result, it was shown that L-glutamate in solution, as in EGTA, has the ability to bind calcium ions in intact synaptosomes, which suggests that the L-glutamate is a calcium ion chelator. Pre-incubation with EGTA led to a decrease in fluorescence by 5 %. In the presence of EGTA in the incubation medium, glutamate at concentrations of 10-100 μM dose-dependently increases the fluorescence level by 25-48 %, which is explained by an increase in the concentration of $[Ca^{2+}]_{in}$, caused by glutamate, primarily due to activation of membrane permeability, Ca^{2+} movement into the cell and release of Ca^{2+} from intracellular depots of the cell (Figure 1).

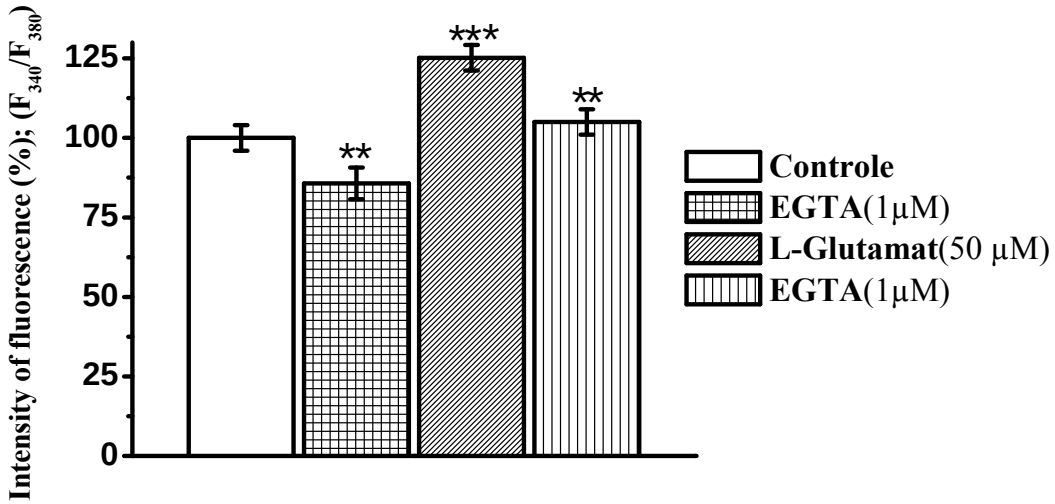

Fig. 1. Effect of L-glutamate (50 μM) on the fluorescence intensity in a suspension of rat brain synaptosomes upon incubation with EGTA (1 μM)
Reliability indicator: * – P < 0.05; ** – P < 0.01; *** – P < 0.001. (n = 6)

It is known that the mechanism of l-glutamate is associated with short-term presynaptic plasticity associated with the release of neurotransmitters and instead determines the form of the response of the postsynaptic neuron and plays a key role in encoding information in the nervous system. This allows the regulation of presynaptic Ca^{2+} channels to either promote or inactivate incoming Ca^{2+} currents. This strong dependence of neurotransmitter release on presynaptic Ca^{2+} current may predict regulatory mechanisms that will affect short-term presynaptic plasticity.

Re-incubation of Caralinia and Granatuum (10-100 μM) with the CTC-synaptosom complex increases the level of $[Ca^{2+}]_{in}$ contrast to glutamate (Figure 2).

A complex effect of Caralinia and Granatuum polyphenols together with L-glutamate on the $[Ca^{2+}]_{in}$ level in synaptosomes was carried out.

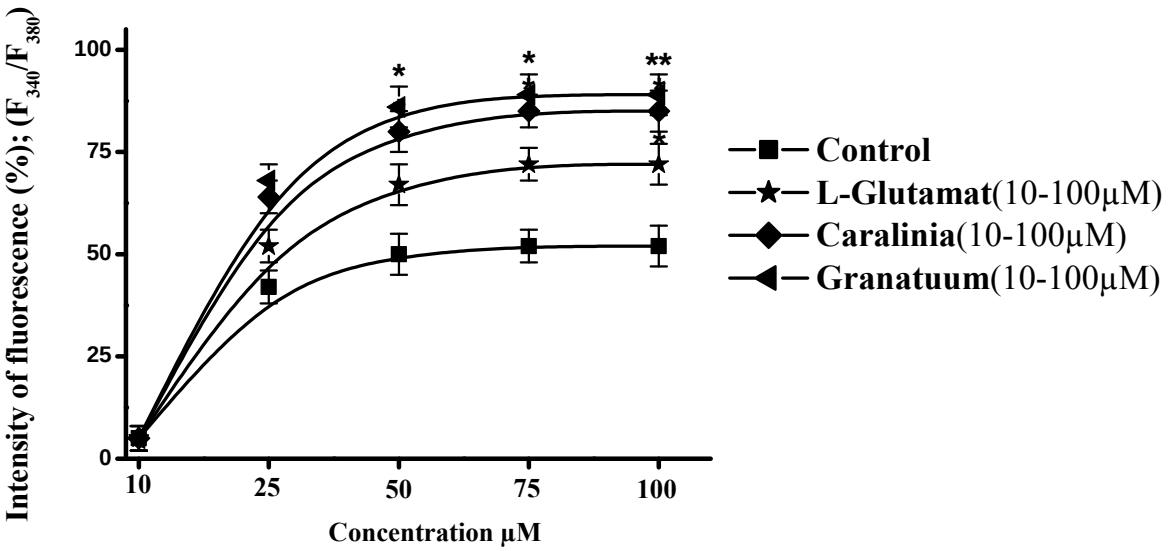

Fig. 2. Effect of Caralinia and Granatuum and L-glutamate at concentrations (10–100 μM) on the intensity of CTC-fluorescence of a rat brain synaptosome suspension.
Reliability indicator:* – P < 0.05; ** – P < 0.01; *** – P < 0.001. (n = 6)

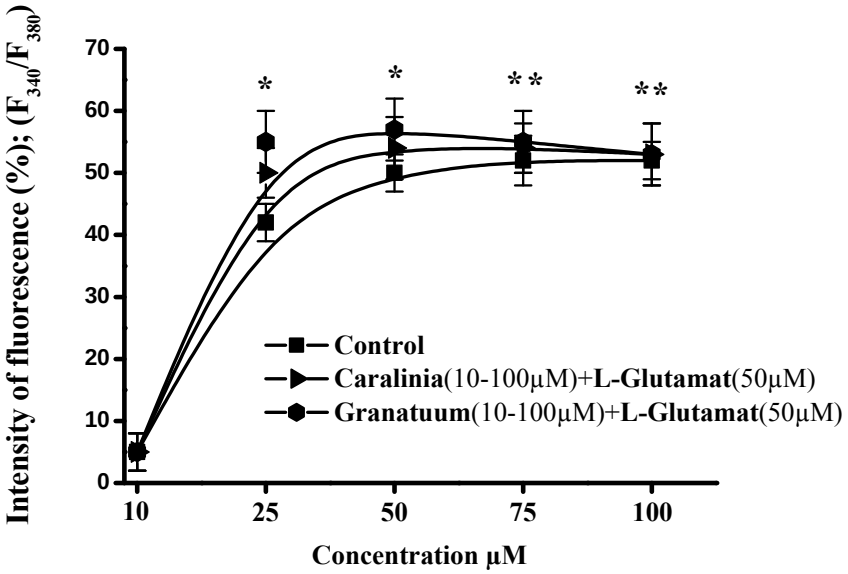

Fig. 3. Effect of Caralinia and Granatuum (10-100 μM) on the fluorescence intensity of a suspension of rat brain synaptosomes incubated with glutamate (50 μM)
Reliability indicator: * – P < 0.05; ** – P < 0.01; *** – P < 0.001. (n = 6).

Preliminary re-incubation of Caralinia and Granatuum (10 μM) with CTC-synaptosomes and then the addition of L-glutamate led to a decrease in $[Ca^{2+}]_{in}$. A dose-dependent and simultaneous increase in the concentration of Caralinia and Granatuum (10-100 μM) led to a dose-dependent decrease in the effect of L-glutamate (Figure 3).

It is known in the literature that activation of the L-glutamate receptor, in turn, causes the opening of calcium channels of ionotropic receptors, the entry of calcium into synaptosomes through membranes and depolarization of membranes, followed by the release of amino acid neurotransmitters (Khoshimov et al., 2018).

The results obtained showed that Caralinia and Granatuum partially reduce the action of L-glutamate, and this gives information that calcium from the outside comes under the influence of caralinin also through the glutamine site and, as a result, open calcium channels in NMDAR.

Further, pre-supplementation with 100 μM glutamate does not completely abolish the effects of Caralinia and Granatuum, suggesting that Caralinia and Granatuum have several mechanisms of action on rat brain neurons leading to increased $[Ca^{2+}]_{in}$.

It was found that the incubation of Caralinia and Granatuum (10–50 μM) in a suspension of synaptosomes significantly increases the intensity of CTC fluorescence. And upon preincubation with L-glutamate (50 μM), Caralinia and Granatuum (10–100 μM) significantly reduces the fluorescence intensity.

The addition of glycine (5 μM) to the incubation medium enhanced the glutamate-dependent enhancement of fluorescence by 15-25 %. Mg2+ (50 μM) inhibits L-glutamate-induced release of $[Ca^{2+}]_{in}$ synaptosomal depots. (Figure 4).

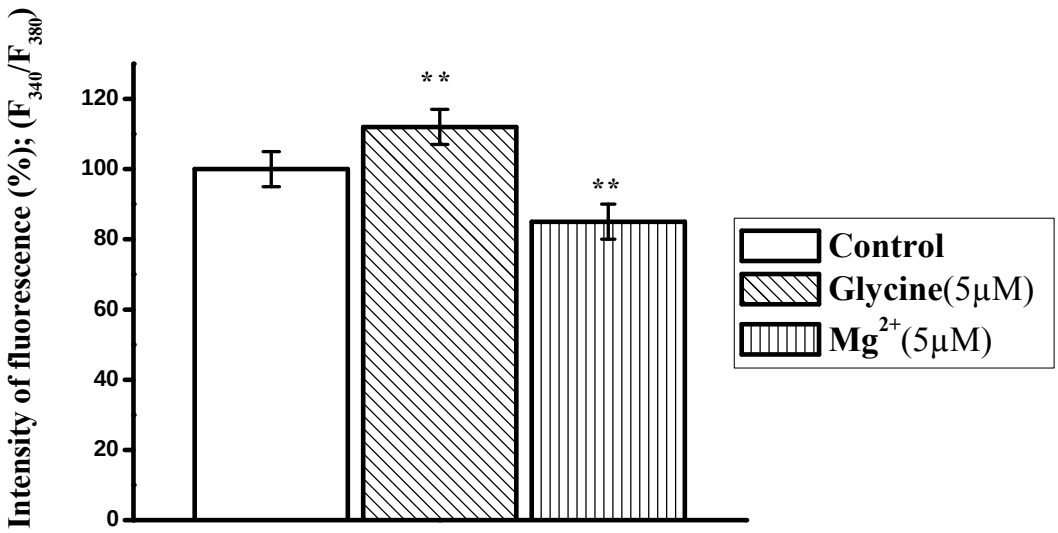

Fig. 4. Effect of glycine and Mg^{2+} ions on glutamate-induced Ca^{2+} intracellular depots.
Reliability indicator: * – P < 0.05; ** – P < 0.01; *** – P < 0.001. (n = 6)

In the following experiments, to identify the possible interaction of Caralinia and Granatuum with NMDAR over excitation sites responsible for opening calcium channels, the effects of Mg^{2+}, argiolobatin and the calcium channel blocker nifedipine were studied.

Experiments have shown that Mg^{2+} at concentrations of 5 mM significantly inhibits NMDAR activity. The inhibitory effect of Mg^{2+} against the background of Caralinia and Granatuum (50 μM) by NMDAR activity did not change.

In the presence of Caralinia and Granatuum, the inhibitory effect of Mg^{2+} (5 μM) did not change. This is probably due to the lack of competition between Mg^{2+} and Caralinia and Granatuum for sites stimulating the opening of Mg^{2+} ion channels.

In place of the action of argiolobatin (1 μM) on calcium channels, NMDAR does not change in the presence of Caralinia and Granatuum (5 μM) (Figure 5).

Incubation of nifedipine (0.01 μM) with the CTC-synaptosome complex resulted in a decrease in fluorescence.

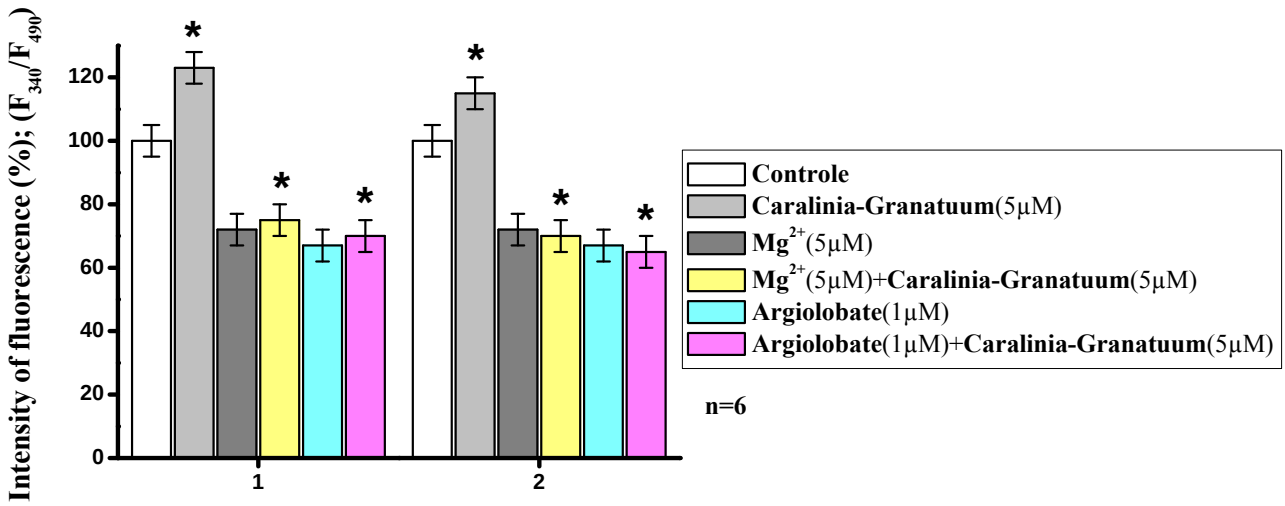

Fig. 5. Effect of non-competitive NMDAR antagonists Mg^{2+} and argiolobatin against the background of 1) Caralinia 2) Granatuum on fluorescence intensity and $[Ca^{2+}]_{in}$ level in rat brain synaptosomes.
Reliability indicator: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 (n = 6).

Pre-incubation of Caralinia and Granatuum (5 μM) against the background of nifedipine (0.01 μM) with the CTC-synaptosomal complex did not lead to a change in fluorescence (Figure 6), which indicates the absence of competition between Caralinia and Granatuum and nifedipine for the site of regulation of dihydropyridine-sensitive calcium channels in synaptosomal membranes.

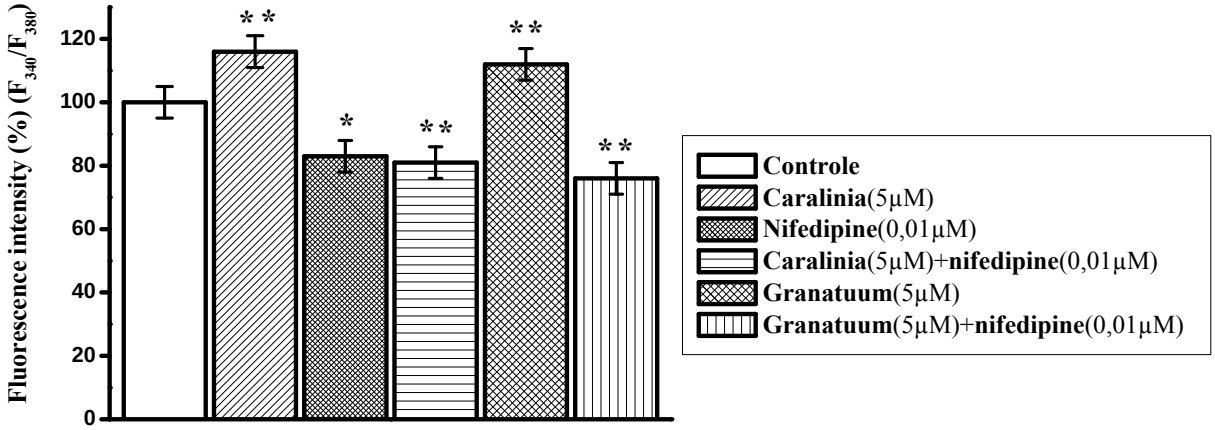

Fig. 6. Effect of Caralinia and Granatuum on calcium-dependent processes of the NMDAR in the presence of nifedipine.
Reliability indicator: * – P < 0.05; ** – P < 0.01; *** – P < 0.001. (n = 6)

Caralinia and Granatuum do not act on dihydropyridine-sensitive calcium channels in the membrane of rat brain synaptosomes.

4. Conclusion

The results obtained show that the Caralinia and Granatuum polyphenols used significantly increased $[Ca^{2+}]_{in}$ levels in synaptic membranes compared to controls. Preliminary results indicate there is a possibility of competition between the polyphenols Caralinia, Granatuum and the L-glutamate antagonist for the NMDAR ion channel opening regulation site.

Under the action of Caralinia and Granatuum polyphenols responsible for opening calcium channels with other NMDAR sites, no changes in the $[Ca^{2+}]_{in}$ level in synaptosomes were observed

against the background of Glycine, Mg^{2+}, argiolobatin and nifedipine. The conclusion of this work makes it possible to create a neuroprojective drug with a therapeutic effect for the treatment of AD based on these polyphenols in pharmacology has good prospects.

5. Acknowledgements

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6. Conflict of interest

No conflict of interest was declared.

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Application of Continuous Glucose Monitoring Systems in Patients With Type 2 Diabetes Mellitus

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Abstract

Though continuous glucose monitoring systems (CGMS) have proven their benefits in type 1 diabetes mellitus, research about their use in type 2 diabetes mellitus (T2D) are still in progress. The current study aimed to evaluate the use of such systems in patients with T2D in comparison with the standard periodic capillary measurements. Twenty-five patients with T2D under insulin or combination of insulin and oral medication participated in a prospective 7-day observational study. American Diabetes Association 2021 targets for good euglycemic control were used as primary goals for the two groups (CGMS over capillary measurements). Seventy two percent of the participant achieved maintenance of blood glucose within therapy target guidelines. Moreover, 64 % of the participants achieved glycohemoglobin HbA1c < 6.5 %.

Glucose values with both methods display high level of correlation ((r = 0.901, p < 0.001) and the same is also valid for HbA1c and estimated HbA1c ((r = 0.939, p < 0.001). Thus, CGMS can achieve both better glycemic control and decrease of HbA1c in patients with T2D. Though the present findings are in accordance with the available literature, further studies in larger populations with more deeper analyses are still needed to confirm the usefulness of CGMS in T2D.

Keywords: continuous glucose monitoring systems, type 2 diabetes mellitus.

1. Introduction

Though COVID-19 pandemic had dominated medical interest in the last 2 years, managing of diabetes mellitus (DM) continues to pose a great challenge. DM global burden constantly rises and prognosis about the future is worsening (IDF Atlas, 2019). Technology advance has created a new branch in DM management that facilitates both monitoring (continuous monitoring systems - CGMS, their flash and total implantable variants) and therapy (with subcutaneous continuous insulin infusionsystems) (Kravarusic et al., 2020). And though CGMS has proven their benefits in type 1 DM (T1D) (Carlson et al., 2017), research about their use in type 2 DM (T2D) are still in

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progress. Results from studies like DIAMOND (Ruedy et al., 2017) or GP-OSMOTIC (Furler et al., 2020) are promising, and systemic reviews are planning for the near future (Zheng et al., 2020).

Within this frame, the current study aimed to evaluate the use of CGMS in patients with T2D in comparison with the standard periodic capillary measurements.

2. Materials and methods

The study took place in Diabetic Unit Care, AHEPA University Hospital, Thessaloniki, Greece from 01/09/2020 – 01/12/2020. Twenty-five (25) patients with T2D under insulin or combination of insulin and oral medication participated in a prospective observational study. Exclusion criteria included: Type 1 diabetes mellitus, specific diabetes types: pregnancy-related, drug or toxin–induced diabetes, endocrinopathy-associated diabetes, patients with acute renal failure or chronic renal failure in hemodialysis, women in reproductive age and any hyperglycemic emergencies (e.g. diabetic ketoacidosis, hyperosmolar hyperglycemic nonketotic syndrome). Informed consent was obtained from all participants.

After full medical history interview and clinical examination, a CGMS sensor (Enlite™ Glucose Sensor, Medtronic SA, Ireland) was applied according to manufacturer's guidelines for a period of 7 days. During study period CGMS recorded 588 measurements in each participant. Along with Glu measurements both from CGMS and capillary blood, CGM parameters (Envision™ Pro CGM System, Medtronic SA, Ireland) such time in range (TIR) (time interval with Glu falling within 70-140 mg/dl), time above range (TAR) (time interval with Glu recordings > 140 mg/dl), time below range (TBR) (time interval with Glu recordings < 70 mg/dl), area under curve (AUC) form Glu values > 140mg/dl and < 70 mg/dl were also recorded. Demographics parameters (age, sex, body weight, BMI), selected blood count parameters (white blood cells-WBC, hemoglobin-Hb, platelets – PLT), glycohemoglobin (HbA1c), urea, creatinine, total cholesterol (Chol) and its high- and low-density fractions (HDL, LDL), lactate dehydrogenase (LDH) and hepatic transaminases (SGOT, SGPT) were also measured. American Diabetes Association 2021 targets for good euglycemic control were used as primary goals for the two groups (American Diabetes Association, 2021).

Data analysis was conducted with SPPS® Statistics for Windows, v.24.0 (IBM Corp. Armonk, NY, USA) and included exploratory descriptive analysis, data normality evaluation (using Kolmogorov-Smirnov test), comparison test (Student's t-test or Mann Whitney U test) and correlation coefficient calculation (Pearson coefficient r^2). Statistical significance level was defined as $p < 0.05$.

3. Results

Data from all 25 patients (15 men and 10 women) were included for further analysis, as no problems/complications were reported during the use of CGMS. Women were elder and shorter than men, yet with almost the same BMI (Table 1).

Table 1. Demographics characteristics of the participants. Data are presented in form of mean (standard deviation). Statistical significance $p < 0.05$

Parameters/Patients	All (n = 25)	Men(n = 15)	Women (n = 10)	p
Age (years)	68.3(11.3)	64.1 (10.3)	74.7 (9.9)	0.017
Weight (kg)	84 (25.5)	84 (25)	77 (28.5)	0.091*
Height (cm)	172.2 (10.4)	175 (13)	165 (9.3)	< 0.001*
BMI (kg/m^2)	27.6 (4.2)	27.6 (4.39)	29.42(8.85)	0.495*

*Mann-Whitney U test

Antidiabetic regiment varied among participants: 40 % of them (10/25) received monotherapy (metformin or dipeptidylpeptidase-4 (DDP-4) inhibitors), 24 % (6/25) received

double therapy, 24 % (6/25) received triple drug regiment and 12 % (3/25) received a combination of four drugs. Only 12 % (3/25) received insulin and 16 % (4/25) a combination (Table 2).

Table 2. Detailed presentation of antidiabetic regiment: SGLT2 – Sodium-glucose cotransporter 2 inhibitors, DDP – 4 inhibitors – Dipeptidyl peptidase – 4 inhibitors, GLP-1, Glucagon-like peptide-1 receptor agonists.

Regiment	All	Men	Women
Metformin	7	3	4
Bigouanide	1	0	1
DDP-4 inh.	2	1	1
DDP-4 inh./Metformin	4	2	2
SGLT-2/Metformin	2	2	0
DDP-4 inh./Metformin/SGLT-2	2	2	0
DDP-4 inh./Metformin/Pioglitazone	1	0	1
DDP-4 inh./Bigouanide/SGLT-2	2	0	1
DDP-4 inh./Metformin/SGLT-2/Pioglitazone	1	1	0
DDP-4 inh./Metformin/SGLT-2/Insulin	1	1	0
SGLT-2/Metformin/Insulin/GLP-1	1	1	0
DDP-4 inh./Metformin/SGLT-2/Sulphonylureas	1	1	0

Twenty eight percent (28 % or 7/25) of the participants had diabetic neuropathy, 76 % (19/25) was under antihypertensive therapy and 64 % (16/25) was receiving also antilipidemic drugs. Antihypertensive regiments included β-blockers, hydrochlorothiazides, angiotensin-converting-enzyme (ACE) inhibitors, Calcium channel blockers and ACE 2 receptors blockers. Dyslipidaemia therapy included statins (atorvastatin, rosuvastatin, simvastatin) or statin-ezetimibe and statin-fibrate combinations.

Most laboratory measurements were the same in both men and women, apart from HDL, Hct and liver transaminases (Table 3).

Table 3. Laboratory characteristic of the participants. Presented in mean (standard deviation-sd) form

	All	Men	Women	p
Ur(mg/dl)	39.1 (7.3)	39.5 (7.7)	38.4 (7.1)	0.713
Cr (mg/dl)	0.9 (0.3)	1 (0.2)	1.3 (1.6)	0.451
Chol tot (mg/dl)	150.1 (17.6)	146.4 (13.3)	155.7 (22.3)	0.203

HDL (mg/dl)	42.8 (6.4)	40.5 (6)	46.1 (5.5)	0.028
LDL (mg/dl)	92.8 (19.6)	89.3 (13.4)	97.9 (26.3)	0.294
Try (mg/dl)	137.7 (42.8)	143.1 (47.1)	129.5 (36.3)	0.448
SGOT (U/l)	29.6 (11.1)	34 (9.5)	22.9 (10.1)	0.011
SGPT (U/l)	31 (10.5)	35.7 (7.2)	23.9 (10.9)	0.003
WBC (mg/dl)	6372 (1389.7)	6460 (1611)	6240 (1039.4)	0.707
Hgb (g/dl)	13.2 (0.9)	13.5 (0.7)	12.8 (1.1)	0.091
Hct (%)	40.5 (2.4)	41.2 (1.9)	39.3 (2.6)	0.049
PLT (K/µL)	235.4 (51.2)	232.5 (55.4)	239.9 (46.6)	0.730

CGMS parameters along with HbA1c and capillary blood measurement are displayed in Table 5.

Table 4. CGSM parameters, capillary blood glucose measurement (Glu_c) and HbA1c in form of mean (standard deviation)

Parameter	All	Men	Women	p
HbA1c (%)	6.3 (1.1	6.3 (0.7)	6.1 (0.3)	**0.048** *
eHbA1c (%)	6.5 (1.7)	6.6 (0.9)	6.15 (1.7)	0.091*
Glu $_{CGMS}$ (mg/dl)	140 (48)	142 (26)	129.5 (48.3)	0.115*
Glu $_{CGMS}$ Max	252.7 (60.3)	254 (47)	212 (98)	0.080*
Glu $_{CGMS}$ Min	86 (24.5)	77 (31)	87.5 (21.5)	1*
TIR(%)	70 (50.5)	70 (27)	82 (43.5)	0.062*
TAR (%)	29 (50.5)	30 (27)	18 (45)	0.062*
TBR (%)	0.3 (0.6)	0.46 (0.7)	0.25 (0.7)	0.567*
Total TAR time (min)	2710 (3228)	2880 (4740)	1370 (2593)	**0.040** *
Total TBR time (min)	21.8 (53.1)	27 (59.2)	14 (44.3)	0.361*
Total TIR (min)	6970 (3540)	5990 (4773)	8065 (3521)	0.096*
TAR/day (min)	387.1 (461.1)	411.4(677.1)	195.7 (370.4)	0.096*
TBR time/day (min)	3.1 (7.6)	3.9 (8.5)	2 (6.3)	0.361*

TIR time/day (min)	995.7 (505.7)	855.7 (681.9)	1152.1 (503.4)	**0.041***
AUC >140	7.4 (23.5)	8.5 (10.2)	3.85 (20.8)	**0.031***
AUC <70	0.02(0.04)	0.027 (0.5)	0.013 (0.4)	0.600*
High exceedances (frequency)	13.4 (7.6)	14.9 (7.8)	14.4 (6.4)	0.621
Low exceedances (frequency)	0.96(2.6)	1.7 (3.6)	0.6 (1.8)	0.488
Glu_c Day 1	150 (81)	152 (120)	126.5 (48.8)	0.291*
Glu_cDay 2	148 (36)	152 (66)	134 (31.5)	**0.024***
Glu_c Day 3	144 (55)	148 (87)	124.5 (55)	0.120*
Glu_c Day 4	124 (65)	136 (118)	105 (35.8)	**0.026***
Glu_c Day 5	120 (56)	139 (54)	109.5 (18.3)	**0.021***
Glu_c Day 6	132 (42.5)	138 (75)	106.5 (40.5)	**0.035***
Glu_cDay 7	124 (56)	142 (72)	109.5 (40.5)	**0.037***
7-days Glu_c mean	133.4 (47.79)	144.4 (90.29)	112.86 (38.93)	**0.052***

According to ADA 2021 guidelines, 72 % of the participant achieved good glycemic control. Moreover, 64 % of the participants achieved HbA1c < 6.5 %.

Finally, Glu_{CGMS} and Glu_c values display high level of correlation (($r = 0.901$, $p < 0.001$) and the same is also valid for HbA1c and eHbA1c (($r = 0.939$, $p < 0.001$)

4. Discussion

The most important finding of this study is the success of glycemic control in 72 % and the achievement of HbA1c < 6.5 % in 64 % of participants. These results come as addition to available literature. Though data for T2D patients are more limited than for patients with T1D, there is evidence of greater benefits of CGMS over other measurements, for patients receiving multiple insulin injections or other regimens (Beck et al., 2020; Ehrhardt et al., 2011). In a recent, 52-week randomized trial in patients with T2D treated with various regimens, the mean reduction in HbA1c at 12 weeks was 1.0 % (sd 1.1 %) with CGMS for four cycles of 2 weeks and 5 % (sd 0.8 %) with self-monitoring blood glucose ($p = 0.006$) (Ehrhardt et al.., 2011). Previously, Yoo et al (Yoo et al., 2008) studied 65 patients with poorly controlled T2D in a variety of treatments and the use of CGMS resulted in a 0.7 % reduction in HbA1c in the intervention group compared with the group randomized to self-monitoring blood glucose.

Beck et al (Beck et al., 2020) randomized study evaluated the benefit of CGM use in 158 T2D patients with mean HbA1c of 8.5 % treated using multiple daily injections. Again, HbA1c decreased to 7.7 % in the CGM group over a 24-week period compared to 8 % in the group with usual care. Furthermore, Craciun et al included 28 patients with T2D to evaluate the impact of short-time CGM on glycemic control and found that HbA1c decreased significantly from 8.8 % at baseline to 7.3 % at follow-up ($p < 0.0001$) in the whole group (Cracium et al., 2014).

Limitation of the available findings (most of the studies are single center, with relatively small sample and lack of cohort-of-interest analyses) may decrease their importance; yet a clear trend seems to be forming. Despite the contradictory or unclear effects of CGM use for patients with T2D and the lack of relevant studies, clinical trial results have shown that the use of CGMS not only reduces HbA1c and hypoglycemia but can alleviate the fear of hypoglycemia and anxiety associated with DM and improve quality of life (Kubiak et al., 2016; Patton et al., 2016). Thus, CGMS are gaining ground among patients as they provide significant benefits ,i.e. comprehensive picture of glycemic variability and connection of glucose excursions with meals, exercise, sleep and medication; information that can enhance the management of DM (Danne et al., 2017).Unfortunately, their extremely high relative cost limits their use only to selected clinical scenarios (Vashist et al., 2013). As technology progresses, larger availability of CGMS may also become more accessible.

5. Conclusion

CGMS can achieve both better glycemic control and decrease of HbA1c in patients with T2D. Yet, further studies in larger populations with more deeper analyses are still needed to confirm this finding.

6. Conflict of interests

The authors have no conflicts of interest to declare.

7. Authors' Contributions

A.M conceptualization, design of the study, data recording and analysis, oral presentation, T.A. literature review, final draft writing, D.T. design of the study, supervision, S.C,K.F literature review, supervision. All authors have reviewed and agree with the final manuscript.

8. Funding

None.

Ethical approval and consent to participate.

The study was conducted for obtaining a MSc degree. It was approved by AHEPA University Hospital of Thessaloniki IRB and included in Aristotle University of Thessaloniki, Medical Faculty, Post-Graduate Education Program, Academic year 2020-2021.Informed consent was from all participants.

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Reasons Linked with COVID-19 Vaccine Taking and Tentativeness Amid Medical Doctors in ATBUTH Bauchi Nigeria

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Abstract

Though COVID-19 has been in existence for more than three years, it remains a great public health problem worldwide, causing mortalities, economic and social problems even withing medical doctors. The current study aimed at discerning the main reason why some medical doctors at ATBUTH show reluctance in receiving the vaccine. A cross-sectional study was carried out using questionnaires administered to consenting medical doctors of ATBUTH. A total of 300 were administered, 117 were returned. Random sampling was employed in the selection of participants. Simple Percentages and Means were used to analyze the data. Findings showed 84.11 % of respondents were vaccinated while 15.88 % were not. 19.7 % were fully vaccinated, 57.3 % were partially vaccinated while 17 % were not vaccinated at all. For protection, to avoid travel restrictions, mandatory for work, fear of segregation, others were the reasons for being vaccinated while religious convictions, fear of segregation, conspiracy theories, dearth of information, absence of choice vaccine were the reasons given by respondents for hesitancy to be vaccinated.

Keywords: COVID 19, Vaccination, Vaccine, vaccine hesitancy, health care workers, cross-sectional, endemic, pandemic, epidermic, ATBUTH.

1. Introduction

It's been almost four years after its outbreak, COVID-19 lingers triggering immense public health upheaval worldwide with simultaneous infections, mortality, stern economic and social issues (Allen, Butler, 2017; Cerda, García, 2021; Workforce, 2021; Cerda, García, 2021). The WHO defines vaccine hesitancy as reluctance or refusal to vaccinate despite the availability of vaccines (Eugenia-toledo-romaní et al., 2022; Marzo et al., 2022). This phenomenon is considered as one of the serious threats to global health (Parsons et al., 2022; Malter et al., 2022; Galal et al., 2022). The problem of covid-19 vaccine hesitancy is also quite significant among Medical Doctors (Cerda, García, 2021; UNCTAD, 2020). Though the vaccination has been launched for some time now, but the extent of its acceptance has not been formally studied. Notably, medical doctors are an easily targetable population to be good role models in the community and foster positive public health opinions. It appears that no study has been conducted in ATBUTH to address COVID-19 vaccine acceptance among medical doctors. Hence, the study aimed to assess the acceptance and hesitancy for the COVID-19 vaccine and associated factors among medical doctors in ATBUTH.

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2. Methodology

Study area

Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) is located in Bauchi town, Bauchi State, North Eastern Nigeria and it is located within my immediate community. It is well-equipped with more than 10 clinical and non-clinical departments with the aim of providing standard health care to both indigent and non-indigent patients.

Study design: A cross-sectional study was carried out using a structured questionnaire administered to Medical Doctors.

Study population: Consenting Medical Doctors of ATBUTH Bauchi.

Inclusion criteria: All consenting Medical Doctors of ATBUTH.

Exclusion criteria: Non-consenting Medical Doctors of ATBUTH.

Data collection: Data was collected using a self-administered questionnaire.

Data analysis: Data was analyzed using Microsoft Office tools.

3. Results

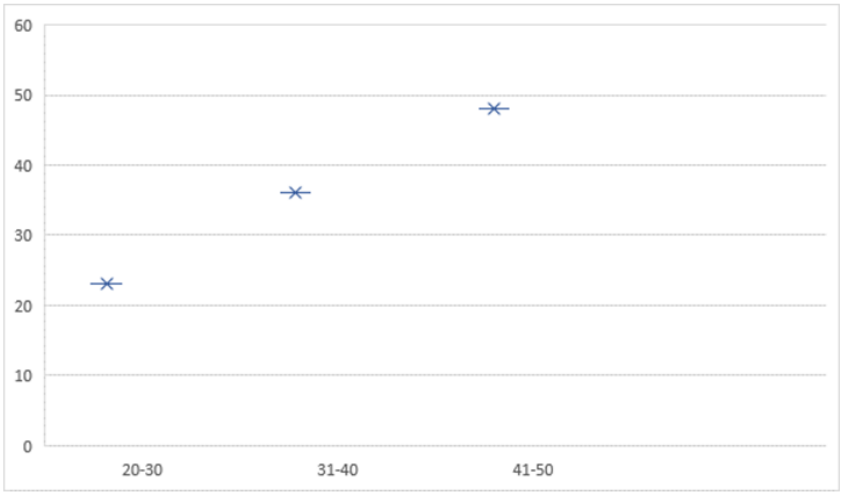

Fig. 1. Distribution of Participants by age

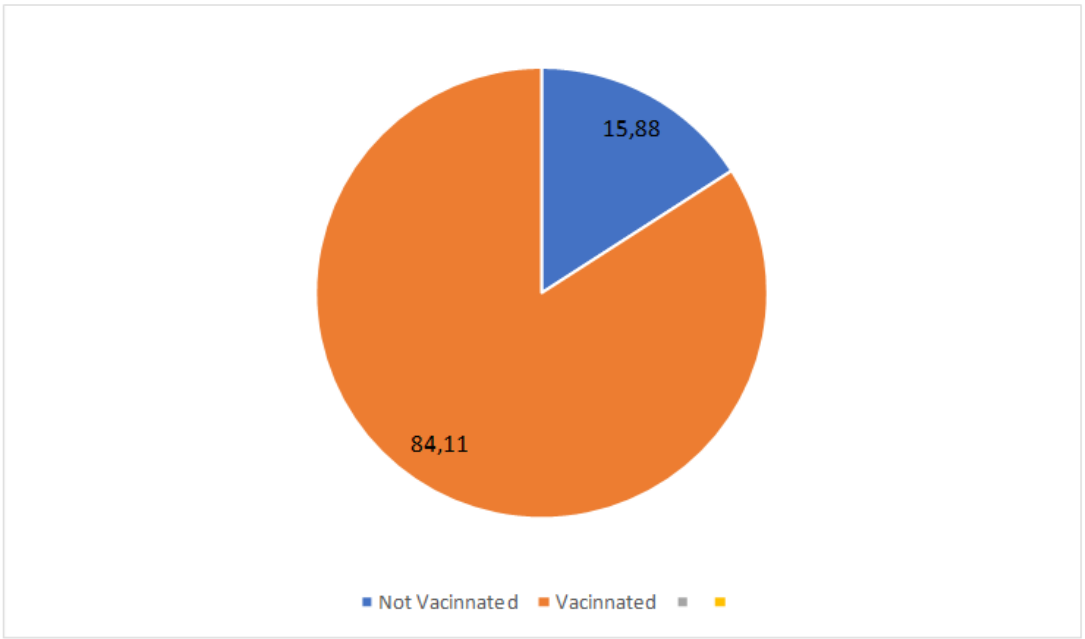

Fig. 2. Percentage Vacinnated

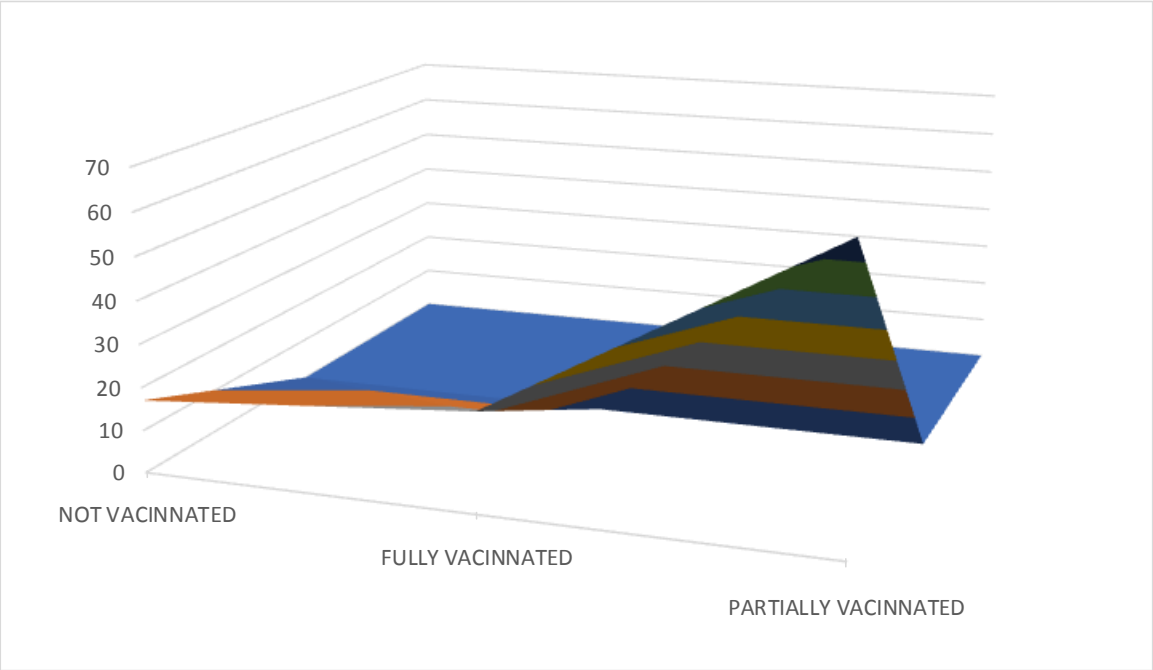

Fig. 3. COVID 19 vaccination status of Participants

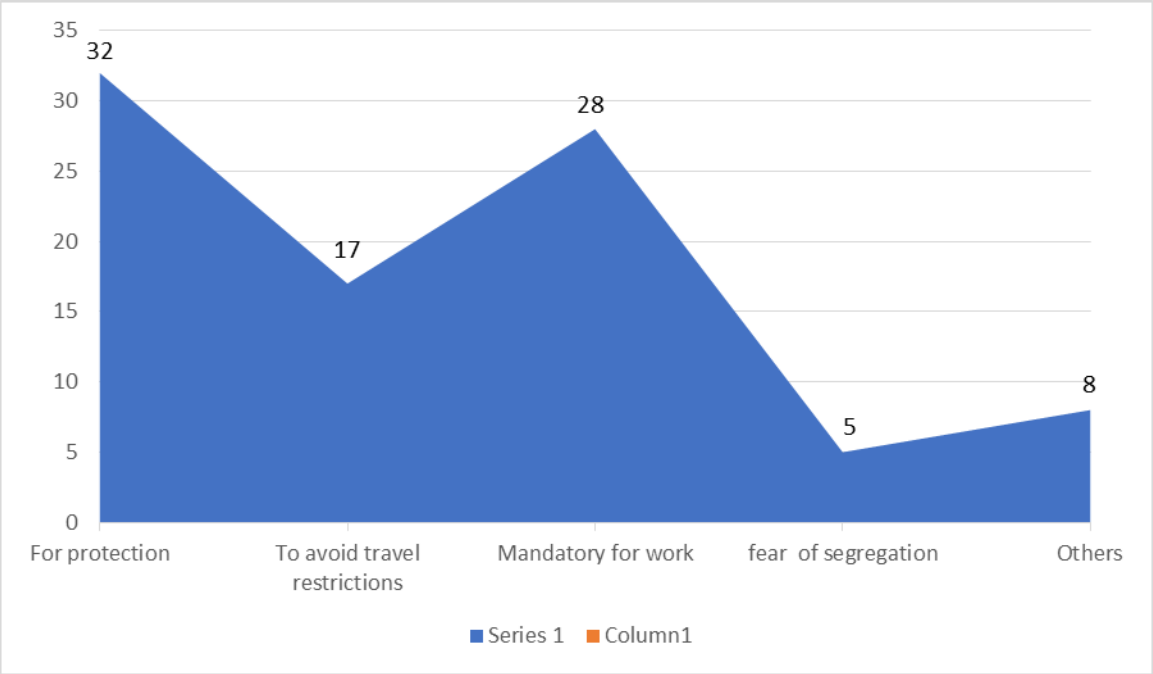

Fig. 4. Reasons for being vaccinated against COVID 19

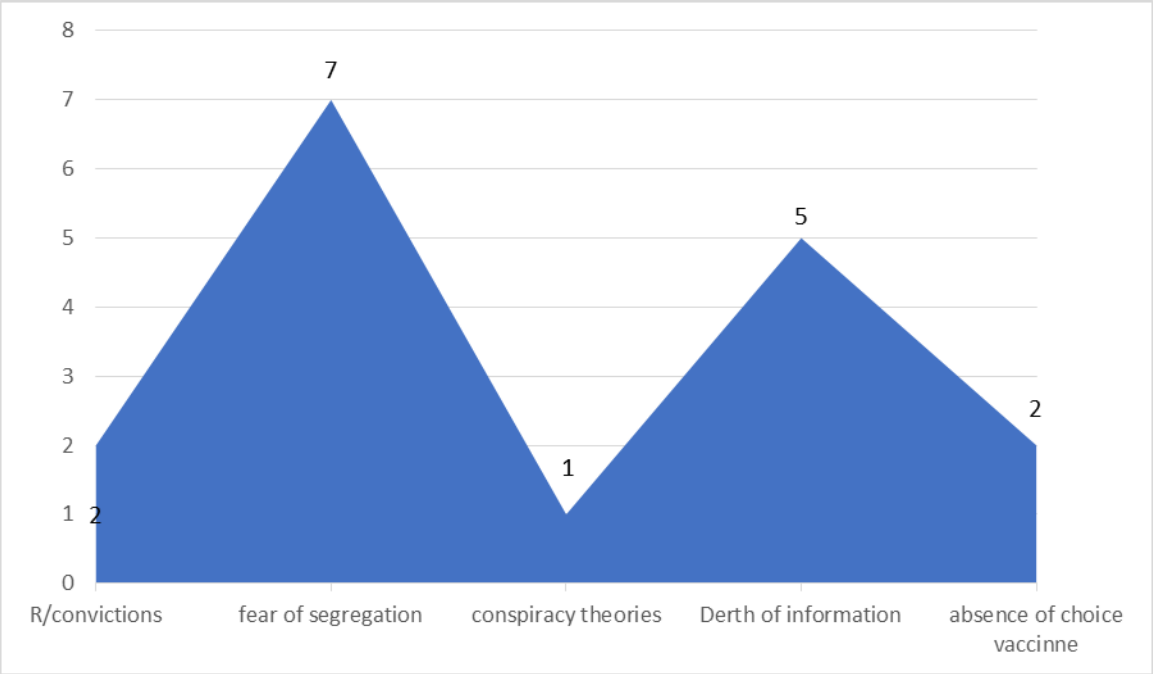

Fig. 5. Reasons for not being vaccinated against COVID 19

4. Conclusion

Findings from this study showed a considerable level of COVID-19 vaccine hesitancy in Medical Doctors, higher than that found in previous studies of Cerda and Garcia (2021) and those of Galal et al, (2022). The proportion of individuals who would refuse to take the vaccine was similar to the prevalence reported by other studies, which has mainly ranged between 2 and 11 % as expounded by Allen and Butler (2017) and Eugenia-toledo-romani et al (2022), although some studies reported that up to 20 % of the partakers were reluctant to take the COVID-19 vaccine Marzo et al. (2022) while another reported that 14 % of the participants were unwilling to take the COVID-19 vaccine due fear of side effects and 11 % because they do not need the vaccine Workforce (2021) and Watanabe (2022). Nevertheless, current study also found that 15.8 % of the participants refuse to take the COVID-19 vaccine. Other studies have explored the uncertainty regarding intention to take the COVID-19 vaccine, which ranged between 10 % and 23 % Pak et al., (2020) and Parsons et al., (2022). However, these studies scrutinized indecision and not hesitancy. While our results are in agreement with earlier studies, our study is tentative and not archetypal of the Medical Doctors population, as only consenting Doctors were sampled.

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Factors Associated with COVID-19 Vaccine Acceptance and Hesitancy amongst other Health Care Workers besides Doctors in ATBUTH Bauchi

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Abstract

The acceptance of COVID-19 vaccine and hesitancy among Health care workers besides doctors in ATBUTH was examined with in order to determine the level of COVID-19 vaccine acceptance among health care workers in ATBUTH and to assess the factors of COVID-19 vaccine non-acceptance/hesitancy among health care workers in ATBUTH. A cross-sectional study was carried out using questionnaires administered to health care workers of ATBUTH. Current studies showed that 70 % of respondents were vaccinated while 40 % were not, reasons given by respondents for accepting the vaccine were: 37 % to avoid travel restrictions, 15 % mandatory by employer, 45 % for protection, while 9 % gave other reasons, those that were hesitant in receiving the vaccine did so for fear of side effects, 35 %, lack of adequate information, 30 %, religious belief, 17 %, while 12 % gave a collection of other reasons. Fear of side effects and limited knowledge about the vaccine appear to be the main reasons for vaccine hesitancy among health care workers in ATBUTH.

Keywords: COVID 19, vaccination, vaccine, vaccine hesitancy, health care workers, ATBUTH.

1. Introduction

More than 89 million COVID-19 cases confirmed and 2 million confirmed deaths were observed worldwide (Workforce, 2021;Halme et al., 2022). The advent of the COVID-19 vaccination was an inspiration of optimism for a return to normal life. Nevertheless, this success is dependent on acceptance and uptake of the vaccine (Pak et al., 2020; Sarıışık, Usta, 2021; Beach et al., 2022). Vaccine-preventable disease epidemics have amplified in recent years (UNCTAD, 2020; Cerda, García, 2021), and there is prodigious public health awareness in monitoring behaviour towards vaccination as well as recognizing influences encouraging vaccine tentativeness and rejection (Allen, Butler, 2017; Soares et al., 2021; Marzo et al., 2022; Galal et al., 2022). Several reasons may be attributed to COVID-19 hesitancy and outright rejection (Soares et al., 2021), they are generally complex and predisposed by many other compounding factors (Sarıışık, Usta, 2021; Beach et al., 2022; Marzo et al., 2022). In light of existing development of various COVID-19 vaccines, the current effort aimed to clarify the obstructions to vaccine uptake amongst other health care workers besides doctors in ATBUTH Bauchi.

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2. Methodology

Study area

Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) is located in Bauchi town, Bauchi State, North Eastern Nigeria and it is located within my immediate community. It is well-equipped with more than 10 clinical and non-clinical departments with the aim of providing standard health care to both indigent and non-indigent patients.

Study design: A cross-sectional study was carried out using a structured questionnaire administered to health care workers.

Study population: Consenting Health care workers besides medical doctors of ATBUTH Bauchi.

Inclusion criteria: All Consenting Health care workers besides medical doctors of ATBUTH Bauchi.

Exclusion criteria: Non-consenting Health care workers besides medical doctors of ATBUTH Bauchi.

Data collection: Data was collected using a self-administered questionnaire.

Data analysis: Data was analyzed using Microsoft Office tools.

3. Results

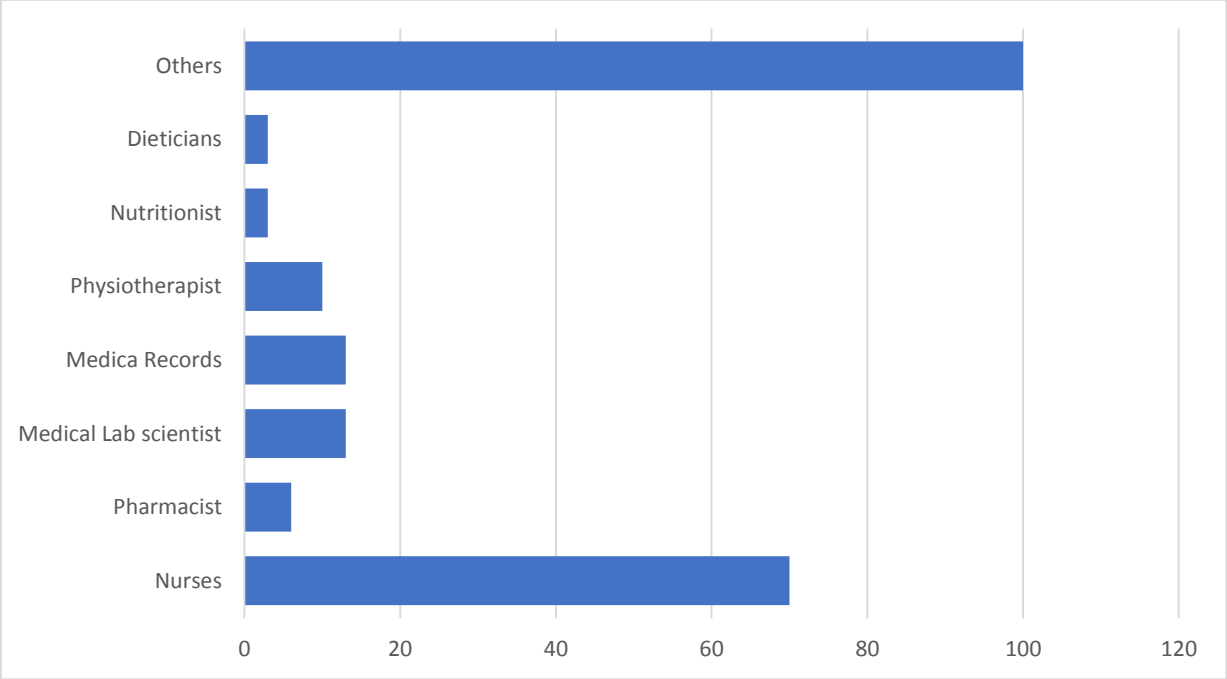

Fig. 1. Distribution of respondents by Cadre

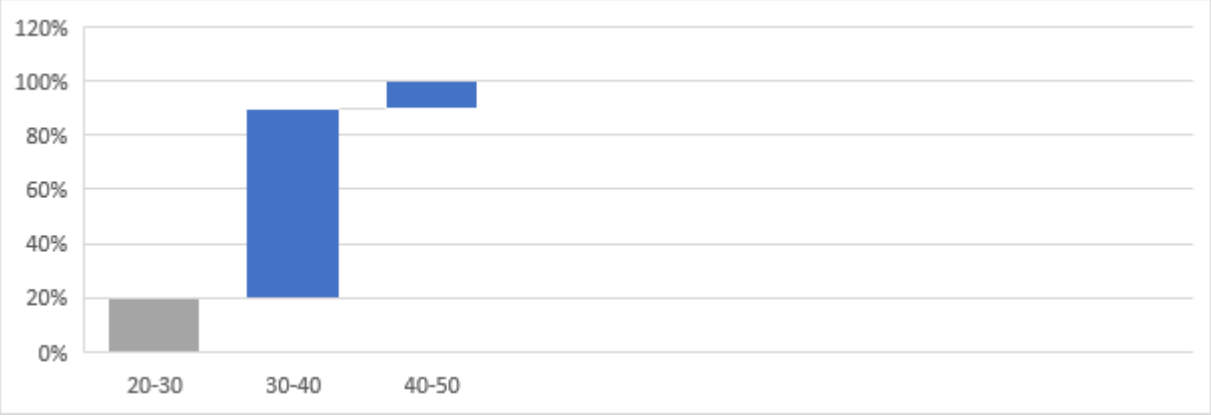

Fig. 2. Percentage distribution of respondents in years

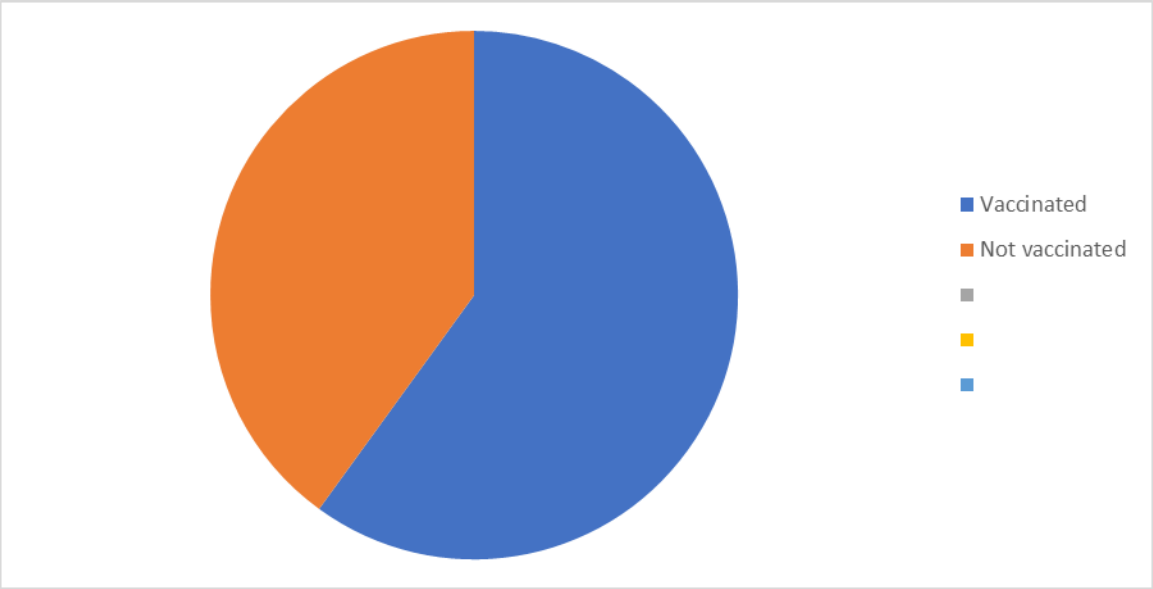

Fig. 3. COVID 19 vaccination status of respondents

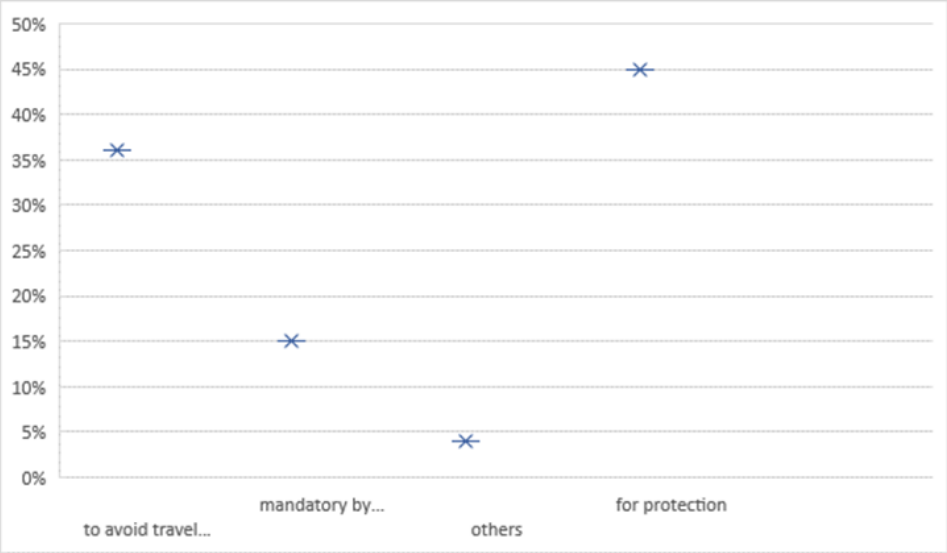

Fig. 4. Reasons for being vaccinated against COVID 19

4. Discussion

Vaccine hesitancy results from a multifaceted decision-making procedure, prejudiced by an extensive variety of circumstantial, distinct and cluster, vaccine-specific factors, together with communication and media, past stimuli, religion, culture, gender, and socioeconomic status, politics, geographic barricades, understanding with vaccination, peril acuity, and design of the vaccination sequencer. Current studies showed that 70 % of respondents were vaccinated while 40 % were not, this is in tandem with studies done by UNCTAD (2022). Reasons given by respondents for accepting the vaccine were: 37 % to avoid travel restrictions, 15 % mandatory by employer, 45 % for protection, while 9 % gave other reasons, though the percentages differ, the reasons were similar to those deduced from studies conducted by Marzo (2022). Those that were hesitant in receiving the vaccine did so for fear of side effects, 35 %, lack of adequate information, 30 %, religious belief, 17 %, while 12 % gave a collection of other reasons which were similarly reported by Pak (2020) and Halme (2022). Recently, there is an increase in vaccine-preventable diseases, hence there is an urgent need in monitoring attitudes towards vaccination as well as recognizing influences contributory to vaccine tentativeness and rejection.

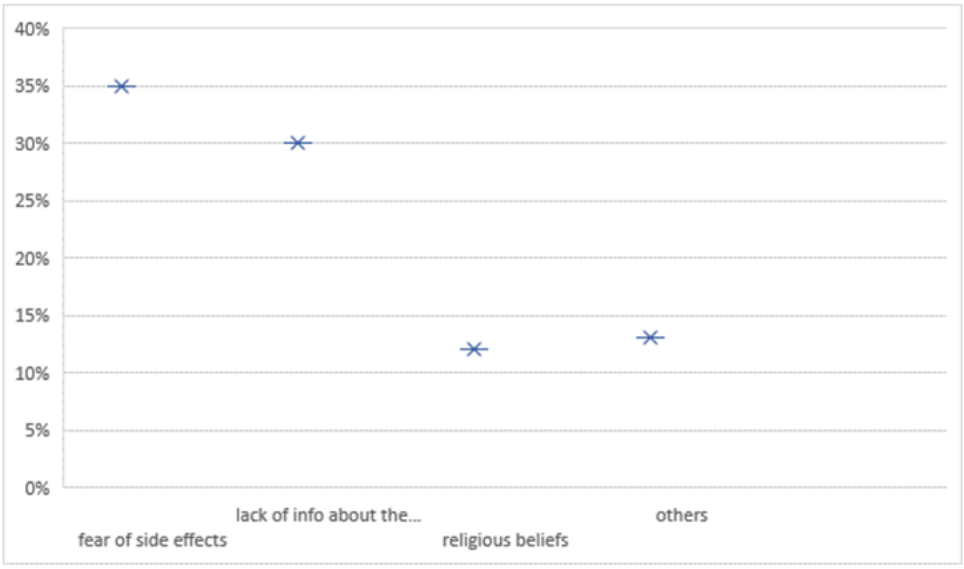

Fig. 5. Reasons for not being vaccinated against COVID 19

5. Conclusion

Covid-19 vaccine hesitancy rates are high worldwide. Strategies need to be adopted to improve vaccine acceptance, thus helping to curb the pandemic.

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